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(54) Title: CONTRACEPTIVE VACCINE

(57) Abstract

The instant invention is drawn to a sperm surface protein in substantially pure form selected from a human PH30 beta chain protein and a mouse PH30 beta chain proteins. Such proteins are useful as contraceptive vaccines in humans and mice respectively, and for identifying small molecules that will disrupt sperm-egg interaction and fertilization.

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TITLE OF THE INVENTION
CONTRACEPTIVE VACCINE

FIELD OF THE INVENTION

The present invention provides sperm surface proteins and DNA sequences encoding the proteins which are useful in the prevention of fertilization. More particularly, the cloning and characterization of the mouse and human PH30 beta chain genes, as well as their use as contraceptive vaccines, are described.

BACKGROUND OF THE INVENTION

Four methods of family planning are currently available in the U.S., sterilization, abstinence, abortion and contraception. Of these four birth control methods, contraception is the most widely utilized. Despite the substantial U.S. and global demand for contraception, the presently available methodologies fall short of market needs. Oral contraceptives and barrier methods dominate today's contraceptive market but have significant shortcomings. Oral contraceptives, though efficacious, are documented to be associated with significant side effects including increased risks of cardiovascular disease and breast cancer and are not recommended for women over the age of 35. Barrier methods, while safe, have failure rates approaching 20%. There is a clear need for increased availability of and improvements in contraceptives that offer superior safety, efficacy, convenience, acceptability and are affordable to women and men worldwide. Identification of novel approaches for controlling fertility is therefore necessary.

Immunization of male and female animals with extracts of whole sperm is known to cause infertility. [Tung, K., et al., *J. Reproductive Immunol.*, 1; 145-158 (1979); Menge, A., et al., *Biol. of Reproduction*, 20, 931-937 (1979)]. Moreover, men and women who spontaneously produce antisperm antibodies are infertile, but otherwise healthy. [Bronson, R., et al., *Fert. and Sterile*, 42, 171-183 (1984)].

Although the critical sperm antigens are unknown, these observations have led to the proposal that sperm proteins might be useful in the development of a contraceptives vaccine.

In mammalian species, sperm proteins are believed to have a role in sperm adhesion to the zona pellucida of the egg. The PH30 protein is known to be involved in sperm egg binding and antibodies that bind to PH30 inhibit this interaction. PH30 is an integral membrane protein present on posterior head of sperm which mediates sperm-oocyte fusion. The PH30 protein consists of two immunologically distinct alpha and beta subunits. Both subunits are made as larger precursors and then finally processed in epididymis where sperm become fertilization competent. [Primakoff, P., et al.,*J. Cell Biology*, 104, 141-149 (1987); Blobel, C.P., et al., *J. Cell Biology*, 111, 69-78 (1990)]. Monoclonal antibodies that recognize PH30 inhibit sperm-oocyte fusion *in vitro*, indicating its importance in fertilization [Primakoff, P., et al., *J. Cell Biology*, 104, 141-149 (1987)].

Guinea pig PH30 alpha and beta chains have been cloned by Blobel et al. Mature PH30 alpha chain consists of 289 amino acids and encodes a transmembrane domain as well as an integral fusion peptide (82-102) that is similar to a potential fusion peptide of E2 glycoprotein of rubella virus. Guinea Pig PH30 beta chain has an open reading frame of 353 amino acids and also encodes a transmembrane domain. [Blobel C.P., et al., *Nature*, 356, 248-251 (1992)]. The predicted amino acid sequence of the PH30 beta chain protein contains significant homology to a class of proteins called disintigrins found in snake venom. These proteins are known to bind to a family of proteins called integrins and prevent their normal functioning in cell adhesion (a well studied example is platelet aggregation). The N-terminal ninety amino acids integrin binding disintigrin domain of PH30 beta has been postulated to mediate the binding of PH30 to its putative integrin receptor on oocytes. The cloning and sequence determination of the mouse and human PH30 beta chain genes would permit novel approaches to the control of sperm

egg binding and fusions. These approaches include, but are not limited to, eliciting an immune response directed at all or part of the PH30 beta chain protein and using the PH30 beta chain protein as part of a screen to identify small molecules that alter sperm egg interactions.

Mammalian fertilization is, in most cases, species specific. Thus, the identification and isolation of sperm surface proteins essential for fertilization in species other than guinea pig would be useful for providing effective long lasting contraception in those species. Thus far, the lack of biochemical identification, isolation and cloning of candidate adhesion proteins of sperm has hindered scientists in developing effective contraceptives for humans as well as other mammalian species.

SUMMARY OF THE INVENTION

The instant invention relates to a sperm protein in substantially pure form selected from a human PH30 beta chain protein, a mouse PH30 beta chain protein or an amino acid sequence substantially homologous to either the human or mouse PH30 beta chain protein.

In one embodiment of the invention is the sperm protein having an integrin binding sequence which is not TDE.

In one class is the sperm protein wherein the integrin binding sequence is selected from FEE or QDE.

In a subclass is the sperm protein which is the human PH30 beta chain protein.

Illustrative of this subclass is the sperm protein having an integrin binding sequence that is FEE.

Further illustrating the invention is a DNA sequence which encodes the sperm protein or a portion of the sperm protein sufficient to constitute at least one epitope.

An illustration is the DNA sequence wherein the epitope is on the native protein.

Exemplifying the invention is the DNA sequence which encodes all or a portion of human PH30 beta chain protein.

An example of the invention is the DNA sequence, wherein the DNA encoding all or a portion of the human PH30 beta protein is characterized by the ability to hybridize, under standard conditions, to the DNA sequence shown in SEQ ID NO: 1.

More particularly illustrating the invention is a contraceptive composition comprising a therapeutically effective amount of the protein, or a polypeptide having the substantially same amino acid sequence as a segment of the protein provided that the polypeptide is sufficient to constitute at least one epitope, and a pharmaceutically acceptable carrier.

Another illustration is the contraceptive composition wherein the epitope is on the native protein.

Further exemplifying the invention is the contraceptive composition, wherein the protein is the human PH30 beta chain protein.

More specifically illustrating the invention is the contraceptive composition, wherein the protein is produced by expressing the gene encoding an immunogenic epitope of the sperm protein in a recombinant DNA expression vector.

Specifically exemplifying the invention is a vector comprising an inserted DNA sequence encoding for the protein.

A further illustration of the invention is the vector, wherein the inserted DNA sequence is characterized by the ability to hybridize, under standard conditions, to a DNA sequence selected from the DNA sequences of SEQ ID NO: 1 or SEQ ID NO: 3.

Another example of the invention is a host that is compatible with and contains the vector.

More specifically exemplifying the invention is a method of producing a human or mouse PH30 beta chain sperm protein, comprising the steps of culturing cells containing PH30 beta chain DNA and recovering the sperm protein from the cell culture.

A further example is the method wherein the DNA encoding all or a portion of the PH30 beta chain protein is characterized by the ability to hybridize, under standard conditions, to a DNA sequence selected from the DNA sequences of SEQ ID NO: 1 or SEQ ID NO: 3.

A more specific illustration is a method of contraception in a human or mouse subject in need thereof, comprising administering to the subject an amount of the sperm protein which is effective for the stimulation of antibodies which bind to the sperm protein in vivo, thereby preventing or substantially reducing the rate of sperm-egg fusion.

Further illustrating the invention is the method wherein the sperm protein has an integrin binding sequence which is not TDE.

Another illustration is the PH30 beta chain protein made by the process described.

Another example is a DNA sequence as shown in Seq. ID No. 1 encoding human PH30 beta chain protein.

Still further illustrating the invention is a purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of human or mouse PH30 beta to allow the possession of the biological property of initiating sperm-egg binding or promoting sperm-egg fusion. This biological activity can be determined using the in vitro sperm-oocyte binding/fusion assays [Primakoff, P., et al., *J. Cell. Biol.*, 104: 141-149 (1987)].

More particularly exemplifying the invention is the DNA sequence wherein the amino acid sequence contains an integrin binding sequence which is not TDE.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram representing the human PH30 beta cDNA gene sequence encoding the human PH-30 beta protein, and the

deduced amino acid sequence of the human PH-30 beta protein present in three letter code. The sequence disclosure of Figure 1 is represented as SEQ ID NO: 1 and 2.

Figure 2 is a diagram representing the mouse PH30 beta cDNA gene sequence, and the deduced amino acid sequence of the mouse PH-30 beta protein present in three letter code. The sequence disclosure of Figure 2 is represented as SEQ ID NO: 3 and 4.

Figure 3 is a restriction MAP of the human PH30 beta cDNA sequence.

Figure 4 is a restriction MAP of the mouse PH30 beta cDNA sequence.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to sperm surface proteins which are essential for fertilization, or portions thereof, and their use in contraceptive methods. A sperm surface protein is essential for fertilization if, for example, a monoclonal antibody to the protein or a polyclonal antibody raised against the purified protein, when bound to sperm, inhibits *in vitro* or *in vivo* fertilization or any step of *in vitro* fertilization. The process of fertilization is defined as the binding or fusion of two gametes (sperm and egg) followed by the fusion of their nuclei to form the genome of a new organism. The surface protein can be located in the plasma membrane of sperm and/or the inner acrosomal membrane. It can be a protein or glycoprotein. The isolated surface protein used for immunization can comprise the entire surface protein or some portion of the protein (external to the cell) which is immunogenic. Two such sperm surface proteins are the mouse and human PH30 beta chain sperm surface proteins. The PH30 beta genes encode proteins which are present on the surface of sperm cells and are essential for fertilization.

As used herein, a protein or peptide is "substantially pure" when that protein or peptide has been purified to the extent that it is essentially free of other molecules with which it is associated in nature.

The term "substantially pure" is used relative to proteins or peptides with which the peptides of the instant invention are associated in nature, and are not intended to exclude compositions in which the peptide of the invention is admixed with nonproteinous pharmaceutical carriers or vehicles.

As used herein, an amino acid sequence substantially homologous to a referent PH-30 beta protein will have at least 70% sequence homology, preferably 80%, and most preferably 90% sequence homology with the amino acid sequence of a referent PH-30 beta protein or a peptide thereof. For example, an amino acid sequence is substantially homologous to mouse PH-30 beta protein if, when aligned with mouse PH-30 beta protein, at least 70% of its amino acid residues are the same. In addition, it is preferable that the substantially homologous amino acid sequence contains the integrin binding sequence.

As used herein, a DNA sequence substantially homologous to a referent PH-30 beta protein will have at least 70%, preferably 80%, and most preferably 90% sequence homology with the DNA sequence of a referent PH-30 beta. Moreover, a DNA sequence substantially homologous to a referent PH-30 beta protein is characterized by the ability to hybridize to the DNA sequence of a referent PH30 beta under standard conditions. Standard hybridization conditions are described in Maniatis, T., et al. (1989) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

An "expression vector" or "vector," as used herein, refers to a plasmid, bacteriophage, virus, or other molecule into which a gene of interest may be cloned, such that the appropriate signals for expression of that gene are present on that vector.

The term "epitope," as used herein, refers to the minimum amount of PH30 beta sequence capable of producing an efficatious, i.e., contraceptive, immune response.

The term "therapeutically effective amount," as used herein, means that amount of a drug or pharmaceutical agent that will elicit the

biological or medical response that is being sought by a researcher or clinician.

Production and Purification of Immunogen

A preferred method for producing sperm surface proteins for use as a contraceptive immunogen is by recombinant DNA technology. To produce the protein using this technology it is necessary to isolate and clone DNA encoding the protein, or an immunogenic portion thereof. Those skilled in the art are familiar with a variety of approaches which can be used in an effort to clone a gene of interest. However, having nothing more than the isolated protein of interest, success in such an effort cannot be predicted with a reasonable degree of certainty.

In the Examples which follow, Applicants describe the cloning and characterization of the mouse and human PH30 beta chain genes. The mouse and human PH30 beta chain genes were isolated using a cDNA encoding the guinea pig PH30 beta chain gene. The instant invention provides specific sequence information to permit targeted intervention in controlling fertility through anti PH30 directed immune responses inhibition of sperm-egg binding and triggering of post binding signaling and effective events. These sequences permit the generation of reagents for the isolation of oocyte proteins involved in sperm-egg interaction.

The information presented in the Examples enable one skilled in the art to isolate and clone the mouse or human PH30 beta chain gene. For example, a cDNA library is prepared from testis or spermatogenic cells isolated from the mammal of interest (e.g., mouse, human). Such a cDNA library is then screened using, for example, labeled guinea pig PH30 DNA probes. DNA encoding all or a portion of human or mouse PH30 is characterized by the ability to hybridize to such a probe sequence under hybridization conditions such as those described in Example 1. Methods of labeling and screening by

hybridization are well known in the art. Positive clones are analyzed, and a full length cDNA is constructed by conventional methods.

The cloned gene, or portions thereof which encode an immunogenic region of the PH30 protein, can be expressed by inserting the coding region into an expression vector to produce an expression construct. Many such expression vectors are known to those skilled in the art. These vectors contain a promoter for the gene of interest as well as additional transcriptional and translational signals. Expression vectors for both eukaryotic host cells and prokaryotic host cells are widely available. The DNA expression construct is used to transform an appropriate host cell.

Eukaryotic, in particular mammalian, host cells are often utilized for the expression of eukaryotic proteins. It has been found, for example, that eukaryotic proteins may exhibit folding problems when expressed in prokaryotic cells. In addition, production of authentic, biologically active eukaryotic proteins from cloned DNA sometimes requires post-translational modification such as disulfide bond formation, glycosylation, phosphorylation or specific proteolytic cleavage processes that are not performed in bacterial cells. This is especially true with membrane proteins. The sperm surface protein is produced using the transcriptional and translational components of the host cell. After an appropriate growth and expression period, the host cell culture is lysed and the sperm surface protein is purified from the lysate. Lysis buffers typically include non-ionic detergent, protease inhibitors, etc.

From the solubilized cell extract, the sperm surface protein can be purified and isolated by physical and biochemical methods such as ultracentrifugation, column chromatography, high performance liquid chromatography, electrophoresis, etc. Alternatively, the sperm surface protein can be isolated by affinity chromatography using monoclonal or polyclonal antibodies [see Primakoff et al., *Biol. of*

Reprod. 38, 921-934 (1988)]. Such methods for purifying proteins are well known to those skilled in the art.

As mentioned above, antigenic portions or epitopes of the sperm surface protein are useful as immunogen, in addition to the full length protein. Antigenic fragments can be produced, for example, by proteolytic digestion of the full length protein, followed by isolation of the desired fragment. Alternatively, chemical synthesis can be used to generate the desired fragment starting with monomer amino acid residues.

With respect to the PH30 protein, certain antigenic domains are preferred candidates for use in a contraceptive vaccine. As is discussed in greater detail in the Exemplification section which follows, the PH30 β subunit contains a domain which is highly conserved when compared to a class of proteins known as disintegrins. A peptide (or portion thereof) which is identical or substantially identical to this domain is preferred for use in the contraceptive methods of this invention. Substantially identical, as used in the preceding sentence, means that at least 70% of the amino acid sequence of the peptide is identical to the corresponding portion of the PH30 β disintegrin domain.

Disintegrins are found in snake venom, for example, and are known to bind to a class of platelet surface proteins known as integrins. The binding of disintegrins to integrins has been shown to inhibit blood clotting. By analogy, peptides corresponding to the PH30 β disintegrin domain are predicted to be active in sperm-egg binding and fusion.

Contraceptive Vaccine

Once the sperm surface protein has been produced and purified, a vaccine can be produced by combining the sperm surface protein or portion thereof with a suitable carrier for administration to a subject for immunization. For successful vaccine development it is

necessary that the immunogen exhibit tissue specificity, that is, it is expressed on the target tissue only and must be essential for the process of reproduction. It is known that the PH30 protein, which is expressed only on sperm, is involved in sperm egg binding and antibodies that bind to PH30 inhibit that interaction.

The cloning and characterization of human PH30 beta permits novel approaches for using PH30 as a target to control human fertility. PH30 beta protein or peptides can be used directly as an antigen to elicit an immune response directed to the whole or a relevant part of the PH30 beta chain protein. Testing of these approaches requires availability of sufficient quantities of PH30 beta protein. The cloning and sequencing of the mouse and human PH30 beta chain provides information necessary to recombinantly express all or part of the PH30 beta protein. These expressed proteins are used with or without adjuvant to immunize women or female mice. The elicited humoral immune responses are monitored by assays that use PH30 beta as antigen. Secreted antibodies in the female reproductive system will bind to the sperm head and disrupt fertilization. The availability of the recombinant mouse PH30 beta protein permits establishment of an animal model system for testing efficacy, reversibility and safety of specific methods of controlling fertility based on PH30.

A vaccine can contain one or more sperm surface proteins. Sperm surface proteins of the present invention can be combined with adjuvants which contain non-specific stimulators of the immune system. Proper use of adjuvants can induce a strong antibody response to foreign antigens (i.e., sperm surface proteins). The action of adjuvants is not fully understood, but most adjuvants incorporate two components. One is a substance designed to form a deposit which protects the antigen from catabolism. Two methods of forming a deposit are to use mineral oils or aluminum hydroxide precipitates. With mineral oils, such as Freund's adjuvant, the immunogen is prepared in a water-in-oil

emulsion. For aluminum hydroxide, the immunogen is either adsorbed to preformed precipitants or is trapped during precipitation.

The second component required for an effective adjuvant is a substance that will stimulate the immune system nonspecifically. These substances stimulate the production of a large set of soluble peptide factors known as lymphokines. In turn, lymphokines stimulate the activity of antigen-processing cells directly and cause a local inflammatory reaction at the site of injection. A component of lipopolysaccharide known as lipid A is commonly used. Lipid A is available in a number of synthetic and natural forms that are much less toxic than lipopolysaccharides, but still retain most of the desirable adjuvant properties of the lipopolysaccharide molecules. Lipid A compounds are often delivered using liposomes. The two bacteria that are commonly used in adjuvants as non-specific stimulants are Bordatella pertussis and Mycobacterium tuberculosis. When used as whole bacteria, they must be heat-killed prior to use. The immunomodulatory mediators of B. pertussis include a lipopolysaccharide component and the pertussis toxin. The pertussis toxin has been purified and is available commercially. M. tuberculosis is commonly found in complete Freund's adjuvant. The most active component of M. tuberculosis has been localized to muramyl dipeptide which is available in a number of forms.

Immunizations (Inoculation and Booster Shots)

The subject to be immunized can be any mammal which possesses a competent immune system. Examples of subject mammals include humans and domestic animals (e.g. dogs, cats, cows, horses, etc.), as well as animals intended for experimental or other purposes (e.g., mice, rats, rabbits, etc.).

Two different criteria are important to consider in determining the proper dose for the initial immunization. First, the optimum dose to achieve the strongest response and second, the

minimum dose likely to induce the production of useful polyclonal antibodies. Much of the injected material will be catabolized and cleared before reaching the appropriate target immune cell. The efficiency of this process will vary with host factors, the route of injection, the use of adjuvants, and the intrinsic nature of the surface protein injected. Thus, the effective dose delivered to the immune system may bear little relationship to the introduced dose and consequently dose requirements must be determined empirically. These determinations can be readily made by one skilled in the art. Secondary injections and later boost can be given with amounts similar to or less than the primary injection.

The route of injection is guided by three practical decisions: 1) what volume must be delivered; 2) what buffers and other components will be injected with the immunogen; and 3) how quickly should the immunogen be released into the lymphatics or circulation. For example, with rabbits, large volume injections normally are given at multiple subcutaneous sites. For mice, large volumes are only possible with intraperitoneal injections. If adjuvants or particulate matter are included in the injection, the immunogen should not be delivered intravenously. If a slow release or the inoculant is desired, the injections should be done either intramuscularly or intradermally. For immediate release, use intravenous injections.

Primary antibody responses often are very weak, particularly for readily catabolized, soluble antigens. Hence, secondary or booster injections are required after the initial immunization. A delay is needed before reintroducing the protein into a primed subject. A minimum of 2 or 3 weeks is recommended but greater intervals are possible. The antibody responses to secondary and subsequent injections is much stronger. Higher titers of antibody are reached, but more importantly, the nature and quantity of the antibodies present in serum changes. These changes yield high-affinity antibodies. The intervals between secondary, tertiary and subsequent injections may also be

varied, but usually need to be extended to allow the circulating level of antibody to drop enough to prevent rapid clearance of newly injected antigen.

Subsequent booster injections will be required to increase reduced circulating antibody for continued contraception. The actual intervals for these injections will differ from species to species. However, the intervals can be determined by one skilled in the art by monitoring serum levels of sperm surface protein antibodies.

In another embodiment, subjects can be administered with alloantisera, or monoclonal antibodies, directed to a sperm surface protein to achieve contraception. The alloantiserum is raised in another individual of the same species, isolated from the serum of the individual and prepared in a suitable carrier for injection into the recipient subject. Those skilled in the art are familiar with methods for preparing and formulating monoclonal antibodies for administration.

There is convincing evidence that naturally occurring antibodies to sperm cause infertility in women [Bronson, R.A., et al., *Fertility and Sterility*, 42: 171-183 (1984)]. This infertility is better correlated with the antibody titers in cervical mucus than with the serum [Clark, G.N., *Amer. J Reprod. Immunol.*, 5:179-181 (1984)]. Presence of anti-sperm antibodies in the cervical mucus of infertile women results in poor sperm penetration through the cervical mucus and agglutination of the sperm, thereby reducing the number of sperm available for fertilization. Thus, success of a contraceptive vaccine depends in particular on the generation of mucosal immune responses involving sustained titers of anti-sperm antibodies in the female reproductive tract.

Generally, local application of the antigen is an effective way to stimulate an antibody response by that mucosa [Mestecky, J., *J Clin. Immunol.*, 7: 265-276 (1987)]. However, local mucosal immunization is ineffective in female reproductive tract due to the barrier function of the luminal epithelium and to rapid loss of antigen from the lumen of reproductive tract. Stability and adhesiveness of the antigen on the mucosal

surface is important for the induction of the mucosal immune responses [de Aizpurua, H.J. and Russell-Jones, G.J., *J Exp. Med.*, 167: 440 (1988)]. Adhesive antigens are critical to successful mucosal immunization, not only because they are effective mucosal immunogens themselves, but also because they are carrier proteins for other antigens. Cholera toxin is a potent immunogen when given mucosally, but acts as an adjuvant when given in combination with other antigens [McKenzie, S.J. and Halsey, J.A., *J. Immunol.*, 133: 1818 (1984)]. Effective immunization is also dependent on the stability of the antigen on a mucosal surface. Many antigens for use in mucosal vaccines are poorly immunogenic because they are unable to survive in the acidic and proteolytic conditions of the mucosal surface [O'Hagen, D.T., *Curr. Opin. Infect. Dis.*, 3:393 (1990)]. The DL-lactide-co-glycolide (DL-PLG) microsphere, microparticle carrier system is one of the most suitable systems for mucosal immunization. DL-PLG microspheres protect the antigen at mucosal surface and are taken up by the mucosal lymphoid tissues where they induce mucosal immunity [Eldridge, J.H. et al, *Curr. Top. Microbiol. Immunol.*, 146: 59 (1989)]. Liposomes and inactivated micro-organisms also are used as microparticle carriers. Some parenteral adjuvants such as Avridine, a lipoidal amine and muramyl dipeptide (MDP), the active component of mycobacteria in Freund's complete adjuvant, also have been shown to be active as oral mucosal adjuvants and enhance mucosal immunization [Anderson, A.O. and Reynolds, J.A., *J. Reticuloendothel. Soc.*, 26(suppl): 667 (1979); Taubman, M.A., et al., *Ann. NY Acad. Sci.*, 409: 637 (1983)]. Development of mucosal immune responses in female reproductive tract are optimized by using various adjuvants, micro particle carriers, by immunizing at local or remote mucosal surfaces or by combination of parenteral and mucosal immunization.

Utility of PH30 beta in Identification of Small Molecules that will Disrupt Sperm-egg Interaction and Fertilization

The comparison of the protein sequences of both mouse and human PH30 beta chain genes shows significant homology to a class of proteins called disintegrins found in the snake venoms. These proteins are known to bind a family of cell surface molecules called integrins and prevent their normal function in cell adhesion. On the basis of these homologies it is reasonable to conclude that the PH30 receptor on the oocyte is an integrin. Comparisons of the disintegrin domain sequences of guinea pig, mouse and human PH30 beta chain genes show significant differences in their putative ligand binding domain. In particular, the sequences in this region are different from other disintegrins and among the three species. The recombinant mouse and human PH30 beta proteins are used to make affinity resins to purify, identify and characterize mouse and human PH30 receptors. The recombinant PH30 beta also are used to determine its relative affinity to other integrins expressed in other tissues and are used as a ligand for cloning of the PH30 receptor.

Since the integrin recognition sequences in PH30 beta are species specific, the sequence information is necessary to identify small molecules that disrupt fertilization in a species specific manner. The recombinant mouse and human PH30 beta are used to set up screens to identify small molecules that act either as antagonist to PH30 receptor and disrupt PH30 binding or act as an agonist and stimulate PH30 receptor inducing transmembrane signaling, egg cortical granule release and zona reaction thus making the egg impenetrable for fertilization.

The present invention is further illustrated in the following exemplification.

EXAMPLE 1

Isolation of DNA Encoding Mouse and Human PH30 beta

A. cDNA Library Plating

One million independent recombinant bacteriophage from both a human testis cDNA library in λgt 11 (Clontech, Palo Alto, CA.) and mouse testis cDNA library (Stratagene La Jolla, CA.) in UNI-ZAP XR were plated. Plaque lifts were done in duplicate by placing a nitrocellulose filter on the plate for two minutes, and treating the filter with denaturing solution (0.5M NaOH, 1.5M NaCl), neutralization buffer (0.5M Tris pH 7.5, 1.5M NaCl) and 2X SSC (3M NaCl, 0.35M sodium citrate pH 7.0) for two minutes each. The filters were dried for thirty minutes at room temperature and then baked for two hours at 80°C in a vacuum oven.

B. Generation of Probe:

A guinea pig PH30 beta cDNA was isolated by RT-PCR (reverse transcriptase-polymerase chain reaction) as a 1020 bp (base pairs), HindIII/Bam HI fragment, containing 94% of the coding sequence. This fragment was subcloned into pBluescript SK^+ vector (Stratagene, La Jolla, CA) and verified by sequence analysis. A probe was made by nick translating the purified 1020 bp guinea pig PH30 beta fragment. The filters were probed at 42°C for fifteen hours in hybridization solution (7mM Tris pH 7.5, 40% formamide, 4X SSC, 0.8X Denhard's, 20 µg/ml of salmon sperm DNA and 10% Dextran sulfate) containing 10^6 cpm (counts per minute)/ml of the labeled probe. The filters were washed twice at room temperature for fifteen minutes each with 2X SSC/0.2% SDS (sodium dodecyl sulfate), then twice at room temperature with 0.2X SSC/0.1%SDS, then once at 42°C for 30 minutes with 0.1X SSC/0.1%SDS. The filters were exposed to XAR film (Eastman Kodak Co, Rochester, NY) for 15 hours. The positive plaques were picked into 1 ml of SM (0.1M NaCl, 10mM Magnesium Sulphate, 2% gelatin, 50mM Tris pH 7.5) and screened again as described above. After four rounds of screening, the purified plaques were obtained.

Purified plaques of mouse testicular library were subcloned into pBluescript SK+ vector using the EX ASSIT helper phage and SOLR cells (Stratagene, La Jolla, CA). DNA from the purified plaques of human testicular library was isolated using light PLG 2 tubes and following manufacturer's (Clontech, Palo Alto, CA) directions. The DNA was then digested with the restriction enzyme EcoRI and ligated into pBluescript SK+ and was used to transform competent E. coli strain HB101 cells.

C. DNA Sequencing and Analysis:

Cloned inserts were sequenced on both strands using the Sequenase kit (United States Biochemical, Cleveland, Ohio). Sequences were analyzed by searching GeneBank and EMBL DNA sequence database using the FASTA program (University of Wisconsin, Genetics Computer Group) and sequence comparisons were done using the GAP program.

D. Characterization of cDNA Clones:

The screening of the mouse testicular library with a 1020 bp guinea pig PH30 beta probe resulted in the isolation of a 1.7 kb (kilo base pair) cDNA clone. This cDNA clone contains a 1371 nucleotide open reading frame and a 329 nucleotide 3' untranslated region. When mature parts of the guinea pig and mouse PH30 beta were compared, the mouse PH30 beta clone showed a maximum of 63% identity to guinea pig PH30 beta at the nucleotide level. The amino terminal 103 residues of the deduced 457 amino acid sequence represents the precursor regions of the mouse PH30 beta that are cleaved off at sperm maturation. At the amino acid level the mature mouse, and guinea pig PH30 betas were 54% identical with all the cysteines lining up.

The human testicular cDNA library screening identified a 2.331 kb cDNA which contains an open reading frame of 1959 nucleotides and 372 nucleotide 3' untranslated region. The human PH30

beta clone was 63 and 67% identical in its open reading frame to mouse and guinea pig PH30 beta genes, respectively. Comparison of the derived 653 amino acid sequence with the mouse and guinea pig PH30 beta indicates that the amino terminal 299 represents the precursor and carboxy terminal 354 amino acids represent the mature part of human PH30 beta respectively. The amino acid sequence of the mature human PH30 beta was 54% homologous to mature guinea pig and mouse PH30 beta proteins.

Protein sequence comparison of mouse and human PH30 beta to guinea pig PH30 beta and snake venom disintegrins indicated significant homology. This analysis revealed similar structural organization and indicated the presence of metalloprotease and disintegrin domains in these proteins.

Metalloprotease domains of mouse and human PH30 beta shared significant similarity with the metalloprotease domains of guinea pig PH30 beta but less similarity to the metalloprotease domain of guinea pig PH30 alpha or other disintegrins. The active site signature sequence of zinc-dependent metalloproteases is present in PH30 alpha and the snake venom disintegrins, Jararhagin and Trigramin. [Wolfsberg, T.G., et al., Proc. Natl. Acad. Sci. USA 90: 10783-10797 (1993)]. Similar to guinea pig PH30 beta, the mouse and human metalloprotease domain lacks the active site signature sequence and both were 80% identical to guinea pig PH30 beta and only 30% identical to guinea pig PH30 alpha metalloprotease active site sequence. Human and guinea pig PH30 beta metalloprotease domains were 60% identical.

Similar to guinea pig PH30 beta, the mouse and human PH30 beta also contain a disintegrin domain. The disintegrin domain in mouse PH30 beta contains 91 amino acids (residues 111-202) and in human, 93 amino acids (residues 299-392). Most disintegrins of snake venom contain a consensus integrin binding sequence RGD. Another family of snake venom disintegrins that are linked to a carboxyl terminus cysteine rich domain, lack the RGD tripeptide but contain a

unique tripeptide and adjacent cysteine. Guinea pig, mouse and human PH30 beta proteins also do not contain RGD tripeptide and share more similarity with this later family of disintegrins. These snake venom disintegrins and disintegrin domains of guinea pig, mouse and human PH30 beta contain a negatively charged residue at the carboxyl end of the tripeptide sequence. The integrin binding sequence of guinea pig PH30 beta is TDE. One skilled in the art would have expected that the integrin binding site of PH30 beta of other mammalian species would also be TDE. However, after isolation of human and mouse PH30 beta, it was found that this was not the case. It was unexpectedly discovered that the critical sequence at the integrin binding site was not conserved. Comparisons of guinea pig, mouse and human PH30 beta disintegrin domains showed significant variation in their putative integrin binding sequences although the carboxy terminus end of these domains were identical. The putative integrin binding residues in PH30 beta were QDE in mouse and FEE in human. These differences in the integrin binding sequences between species were an unexpected and surprising finding.

Both mouse and human PH30 beta contain an epidermal growth factor like repeat and a transmembrane domain that are 60% identical to similar regions of guinea pig PH30 beta.

EXAMPLE 2

Cloning of the 5' end of Mouse and Human PH30 Beta

The 5' ends of mouse and human PH30 beta were cloned using the Gibco BRL "5' RACE System for Rapid Amplification of cDNA Ends" and following manufacturer's protocols. 2 oligonucleotides were synthesized for each template. Oligo 1 was an antisense primer and Oligo 2 was also an antisense primer, internal to oligo 1, and contained in the CAU sequences on the 5' end to facilitate cloning. Oligo 1 was annealed to mouse or human testis mRNA and a

cDNA copy was made using SuperScript II Reverse Transcriptase. The mRNA template was degraded with Rnase H. The single strands cDNA copy was purified with GlassMAX Spin columns and was then tailed on the 3' end with dCTP and terminal transferase. The tailed cDNA copy was then amplified using a supplied anchor primer that contains the 5'CAU cloning site and oligo 2. The amplification system was Taq polymerase. The amplified product was then gel purified, treated with Uracil DNA Glycosylase, subcloned into the vector pAMP1 and then transformed into competent E. coli DH5 cells. Colonies were identified which had subcloned fragment and these colonies were sequenced as described previously.

The complete mouse cDNA sequence and the deduced amino acid sequence of the mouse PH30 beta protein is shown in SEQ ID NO: 5 and SEQ ID NO: 6. The complete human cDNA sequence and the deduced amino acid sequence of the human PH30 beta protein is shown in SEQ ID NO: 7 and SEQ ID NO: 8.

At the nucleotide level, the complete human PH30 beta shares 68% identity with mouse and 68.6% identity with guinea pig PH30 beta, respectively. Mouse and guinea pig DNA sequences are 65.5% identical. The amino acid sequence of the human PH30 beta is 58.9% identical to mouse and 56.5% identical to guinea pig PH30 beta. At the amino acid level, the mouse and guinea pig PH30 beta are 55.2% identical.

EXAMPLE 3

Contraceptive Vaccination by the Administration of PH30 beta Protein

Female or male mice (about 7 weeks old at the time of first injection) receive two injections of PH30 beta in the amounts stated below. Recombinant or native PH30 beta, purified from cell line or sperm by mAb-affinity chromatography or biochemical methods, shows at least 90% purity (i.e., no more than 10% detectable contaminants) using silver-staining of purified protein on SDS gels. Purity of each

PH30 preparation used for immunization of females or males is verified by SDS polyacrylamide gel electrophoresis and silver staining. The affinity-purified PH30 beta, in 0.375 ml phosphate-buffered saline (PBS) containing 3 mM octyglucoside (OG) is emulsified with 0.375 ml complete Freund's adjuvant (CFA). Each animal receives 0.1 ml of the emulsion subcutaneously in the back and 0.05 ml intramuscularly in a rear leg. About 3 weeks later, the same amount of PH30 beta in PBS and 3 mM OG is emulsified with incomplete Freund's adjuvant (IFA), and is injected in the same sites in each animal. Control females and males receive the same injections on the same schedule and containing PBS and 3 mM OG and CFA or IFA, but lacking PH30 beta. To allow the injected females to mate, about 6 weeks after the initial injection they are housed with males for 10 days. Each cage contains one male (13 weeks old) , one PH30 beta immunized female, and from 2-4 control injected females. 24 hours after the grouping, females are checked visually daily for the vaginal plugs. Two weeks after the initiation of the mating the, females are removed into individual cages. After three weeks the pregnant females having litters and progeny are counted. To allow the injected males to mate, about six weeks after the initial injection, each injected male is housed with two females (10-13 weeks) for 10 days. The females and males are then separated and after an additional 3 weeks pups are counted.

EXAMPLE 4

Use of PH30 Disintegrin Peptides as Inhibitor of Sperm Fusion to Egg Plasma Membrane

Peptides from the PH30 β disintegrin domain are tested for inhibition of sperm binding to the egg plasma membrane.

The fusion inhibition assay is carried out as follows. Young female mice (8-10 weeks of age) are injected with 5 units of pregnant mare's serum (PMS) in 0.9 NaCl intraperitoneally. 48 hours later, the

mice are injected IP with 5 units of hCG (human chorionic gonadotrophin) in 0.9% NaCl to trigger super ovulation. 14-16 hours after hCG injection, the ovulated oocytes are collected and treated with hyaluronidase to remove cumulus cells. The zona pellucida is removed with a mixture of proteases. The zona pellucida free eggs are incubated in culture media with peptide at a specified concentration for 30 minutes [Hogan, B.. et al., *Manipulating The Mouse Embryo*, 91-101, (1986)]. Sperm collected from the epididymis of male mice is capacitated by incubation and acrosome reacted as described by Fleming and Yanagimachi [*Gamete Res.* 4, 253-273 (1981)] and added to the eggs and incubated for 15 minutes. The eggs are then transferred to a sperm free culture medium and incubated for an additional 1 hour and 45 minutes. The eggs are then fixed and stained as described by Primakoff et al., [*J. Cell. Biol.* 104, 141 (1987)]. The total number of swollen sperm heads are then counted. Swollen sperm heads are an indication that the sperm and egg have fused.

On the basis of these observations, several indices are calculated. The fertilization index (F.I.) is determined by dividing the total number of swollen heads by the total number of eggs. The fertilization rate (F.R.) is the percentage of eggs fertilized. The percent inhibition is determined by dividing the fertilization index of the experimental peptide by the fertilization index of the control peptide.

The PH30 β disintegrin domain represents an epitope which is critical in sperm-egg fusion. Antibodies which bind specifically to this epitope block sperm/egg fusion.

EXAMPLE 5

Use of PH30 beta to Identify Small Molecules that will disrupt sperm-egg Interaction and Fertilization

A. Identification of PH30 beta receptor antagonists:

Identification of compounds that specifically interfere with the binding of PH30 to their receptor on the egg, has been limited due to unavailability of the sufficient quantities of PH30 protein and normal human eggs. The availability of the rPH30 beta facilitates the identification and cloning of PH30 beta receptor integrin cDNAs. These PH30 beta receptor cDNAs are used to generate recombinant PH30 beta receptors. The alternative source of PH30 beta receptors facilitates identification of substances that affect the binding of PH30 beta to its receptors.

Using conventional methods, the Chinese Hamster Ovary cells are transfected with cDNAs encoding the PH30 beta receptor to produce a stable transformed cell which expresses human PH30 beta receptor integrin in large quantities. Such a transformed cell provides a consistent source of recombinant PH30 beta receptors and is useful in the characterization of the binding of PH30 beta to its receptor and for establishing assays to screen for compounds that inhibit PH30 binding to its receptor.

Selectivity of the compounds to PH30 beta receptor is examined by using cell lines that express other integrin receptors that contain the same beta subunit and closely related alpha chain. Compounds that specifically inhibit PH30 beta/receptor interaction are tested further in biological assays, like inhibition of sperm-egg fusion assay and egg cortical granule release assay to determine their efficacy in inhibiting fertilization.

B. Protocol for PH30 beta antagonist screen:

Cells expressing PH30 beta receptor are treated with extraction buffer (50 mM Tris pH 7.6, 100 mM n-Octyl β-D-Glucopyranoside, 150 mM NaCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$) and soluble material is separated by centrifugation and stored frozen at -80 °C. In an assay tube the 15 µl water, 80 µl of assay buffer (125 mM Tris pH 7.6, 187.5 mM NaCl, 1.25 mM $CaCl_2$, 1.25 mM $MgCl_2$ and 1.25%BSA) and 5 µl of sample compound or control (40 µM of cold PH30 beta) are added and mixed with 50 µl of ^{125}I-PH30 beta (final concentration 40 pM) and 50 µl of cell extract (final protein concentration 250 µg/ml). The tubes

are incubated at room temperature for 1 hour. Following incubation the samples are harvested using Tomtec Mach II- 6x 16 cell harvester and printed filtermat cat. # 1205-404. Filters are dried and counted in LKB/Wallac Beta Plate counter.

Calculations and Interpretations:

$$\% \text{ Inhibition} = \frac{\text{CPMavg total binding - CPMavg sample}}{\text{CPMavg total binding - CPMavg positive control}} \times 100$$

When % inhibition > 60 and the inhibition is dose related, the sample will be considered active.

C. Sperm-Oocyte fusion assay:

Young female mice (approximately 8-10 weeks of age) are injected with 5 units of pregnant mare's serum (PMS) in 0.9 NaCl intraperitoneally. 48 hours later, the mice are injected IP with 5 units of hCG (human chorionic gonadotrophin) in 0.9% NaCl to trigger super ovulation. 14-16 hours after hCG injection, the ovulated oocytes are collected and treated with hyaluronidase to remove cumulus cells. Zona pellucida is removed by treating eggs briefly with 0.1 mg/ml of chymotrypsin. Oocytes are washed with Hepes buffered culture medium and are loaded with a fluorescent stain 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) by incubating at 37°C for 30 minutes. Oocytes are then washed with medium and incubated with rPH30 beta or inhibitor compound for 30 minutes followed by another 30 minute incubation with $1x10^4$ sperms that have been previously capacitated by incubating with calcium ionophore. After incubation, the oocytes are washed, mounted and examined by light microscopy and scored for the presence of fluorescent swollen sperm heads with associated tails in cytoplasm.

$$\text{Fertilization rate} = \frac{\text{number of eggs fused}}{\text{number of eggs tested}} \times 100 \quad \text{(results expressed as \% fertilization)}$$

In the absence of any inhibitor > 90% oocytes are fertilized. When the sperm-oocyte fusion is inhibited >60% and the inhibition is dose related the compound will be considered active.

While the invention has been described and illustrated with reference to certain preferred embodiments thereof, those skilled in the art will appreciate that various changes, modifications and substitutions can be made therein without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ALVES, KENNETH
GUPTA, SUNIL K.
HOLLIS, GREGORY F.

(ii) TITLE OF INVENTION: CONTRACEPTIVE VACCINE

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: USA
(F) ZIP: 07065

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: APPOLLINA, MARY A
(B) REGISTRATION NUMBER: 34,087
(C) REFERENCE/DOCKET NUMBER: 19244Y

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (908)594-3462
(B) TELEFAX: (908)594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2373 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TATGGAATAG AACCCCTGGA GTCTTCAGTT GGCTTTGAAC ATGTAATTTA CCAAGTAAAA 180

CATAAGAAAG CAGATGTTTC CTTATATAAT GAGAAGGATA TTGAATCAAG AGATCTGTCC 240

TTTAAATTAC AAAGCGCAGA GCCACAGCAA GATTTTGCAA AGTATATAGA AATGCATGTT 300

ATAGTTGAAA AACAATTGTA TAATCATATG GGGTCTGATA CAACTGTTGT CGCTCAAAAA 360

GTTTTCCAGT TGATTGGATT GACGAATGCT ATTTTTGTTT CATTTAATAT TACAATTATT 420

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AAGATGTGTG ATGCAAACTA TGCAGGAGGT GTTGTTCTGC ACCCCAGAAC CATAAGTCTG 660

GAATCACTTG CAGTTATTTT AGCTCAATTA TTGAGCCTTA GTATGGGGAT CACTTATGAT 720

GACATTAACA AATGCCAGTG CTCAGGAGCT GTCTGCATTA TGAATCCAGA AGCAATTCAT 780

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AAGCAGAAGT CCCAGTGTCT TCACAATCAG CCTCGCTTAG ATCCTTTTTT CAAACAGCAA 900

GCAGTGTGTG GTAATGCAAA GCTGGAAGCA GGAGAGGAGT GTGACTGTGG GACTGAACAG 960

GATTGTGCCC TTATTGGAGA AACATGCTGT GATATTGCCA CATGTAGATT TAAAGCCGGT 1020

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TCAGTTCAAT CAGATCTATG GCCTGGTGGG AGTATTGACA GTGGCAATTT TCCACCTGTA 1740

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ATGAGATGGC CATTTTTCTT ATTCATTCCT TTCTTTATTA TTTTCTGTGT ACTGATTGCT 1860

ATAATGGTGA AAGTTAATTT CCAAAGGAAA AAATGGAGAA CTGAGGACTA TTCAAGCGAT 1920

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TGAGTTTTAC ATTACAAATT TCTGTTTTTT TAAAGTTATC TTACGCTATT TCTGTTGGTT 2220

AGTAGACACT AATTCTGTCA GTAGGGGCAT GGTATAAGGA AATATCATAA TGTAATGAGG 2280

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AAAGGTAGGT AGGTAAAAGA ATTATATTAT CAA 2373

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 651 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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35 40 45

Ser Val Gly Phe Glu His Val Ile Tyr Gln Val Lys His Lys Lys Ala
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Asp Val Ser Leu Tyr Asn Glu Lys Asp Ile Glu Ser Arg Asp Leu Ser
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Phe Lys Leu Gln Ser Ala Glu Pro Gln Gln Asp Phe Ala Lys Tyr Ile
85 90 95

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Glu Met His Val Ile Val Glu Lys Gln Leu Tyr Asn His Met Gly Ser
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Asp Thr Thr Val Val Ala Gln Lys Val Phe Gln Leu Ile Gly Leu Thr
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    130                 135                 140

Glu Leu Trp Ile Asp Glu Asn Lys Ile Ala Thr Thr Gly Glu Ala Asn
145                 150                 155                 160

Glu Leu Leu His Thr Phe Leu Arg Trp Lys Thr Ser Tyr Leu Val Leu
                165                 170                 175

Arg Pro His Asp Val Ala Phe Leu Leu Val Tyr Arg Glu Lys Ser Asn
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Tyr Val Gly Ala Thr Phe Gln Gly Lys Met Cys Asp Ala Asn Tyr Ala
        195                 200                 205

Gly Gly Val Val Leu His Pro Arg Thr Ile Ser Leu Glu Ser Leu Ala
    210                 215                 220

Val Ile Leu Ala Gln Leu Leu Ser Leu Ser Met Gly Ile Thr Tyr Asp
225                 230                 235                 240

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            260                 265                 270

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        275                 280                 285

Asn Gln Pro Arg Leu Asp Pro Phe Phe Lys Gln Gln Ala Val Cys Gly
    290                 295                 300

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Asp Cys Ala Leu Ile Gly Glu Thr Cys Cys Asp Ile Ala Thr Cys Arg
                325                 330                 335

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            340                 345                 350

Leu Phe Met Ser Lys Glu Arg Met Cys Arg Pro Ser Phe Glu Glu Cys
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Tyr Val Gly Lys Phe Leu Leu Gln Ile Pro Arg Ala Thr Ile Ile Tyr
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485 490 495

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Asn Lys Lys His Cys His Cys Ser Ala Ser Tyr Leu Pro Pro Asp Cys
545 550 555 560

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Ile Pro Phe Phe Ile Ile Phe Cys Val Leu Ile Ala Ile Met Val Lys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1768 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GTACCTGTCT GCGTGATGAA CCCGGAAGCG CCTCACTCCA GCGGTGTCCG GGCCTTCAGT 240

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GAAGATGAAA TTTGCGACTG TGGAAAGAAG GGCTGTGCAG AAATGCCCCC GCCATGCTGT 420

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TCGTGCAAGC TGAAGCGGAA AGGGGAGGTT TGCAGGCTTG CCCAAGATGA GTGTGATGTC 540

ACAGAGTACT GCAACGGCAC ATCCGAAGTG TGTGAAGACT TCTTTGTTCA AAACGGTCAC 600

CCATGTGACA ATCGCAAGTG GATCTGTATT AACGGCACCT GTCAGAGTGG AGAACAGCAG 660

TGCCAGGATC TATTTGGCAT CGATGCAGGC TTTGGTTCAA GTGAATGTTT CTGGGAGCTG 720

AATTCCAAGA GCGACATATC TGGGAGCTGT GGAATCTCTG CTGGGGGATA CAAGGAATGC 780

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GACCCCACAT ACTTACCTCC AGATTGTAAA AGAATGAAAG ATTCATATCC TGGCGGGAGC 1140

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GATTTACTTG TAAGAAATGA ATGATTATGA ATTTCATATT ATACTTTGAT ATTTTACCCT 1620

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TAGGTAGGTA AAAGAATTAT ATTATCAA 1768

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Thr Ser Asp Tyr Val Gly Ala Thr Tyr Gln Gly Lys Met Cys Asp
1 5 10 15

Lys Asn Tyr Ala Gly Gly Val Ala Leu His Pro Lys Ala Val Thr Leu
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Glu Ser Leu Ala Ile Ile Leu Val Gln Leu Leu Ser Leu Ser Met Gly
35 40 45

Leu Ala Tyr Asp Asp Val Asn Lys Cys Gln Cys Gly Val Pro Val Cys
50 55 60

Val Met Asn Pro Glu Ala Pro His Ser Ser Gly Val Arg Ala Phe Ser
65 70 75 80

Asn Cys Ser Met Glu Asp Phe Ser Lys Phe Ile Thr Ser Gln Ser Ser
85 90 95

His Cys Leu Gln Asn Gln Pro Thr Leu Gln Pro Ser Tyr Lys Met Ala
100 105 110

Val Cys Gly Asn Gly Glu Val Glu Glu Asp Glu Ile Cys Asp Cys Gly
115 120 125

Lys Lys Gly Cys Ala Glu Met Pro Pro Pro Cys Cys Asn Pro Asp Thr
130 135 140

Cys Lys Leu Ser Asp Gly Ser Glu Cys Ser Ser Gly Ile Cys Cys Asn
145 150 155 160

Ser Cys Lys Leu Lys Arg Lys Gly Glu Val Cys Arg Leu Ala Gln Asp

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Glu Cys Asp Val Thr Glu Tyr Cys Asn Gly Thr Ser Glu Val Cys Glu
            180                 185                 190

Asp Phe Phe Val Gln Asn Gly His Pro Cys Asp Asn Arg Lys Trp Ile
        195                 200                 205

Cys Ile Asn Gly Thr Cys Gln Ser Gly Glu Gln Gln Cys Gln Asp Leu
    210                 215                 220

Phe Gly Ile Asp Ala Gly Phe Gly Ser Ser Glu Cys Phe Trp Glu Leu
225                 230                 235                 240

Asn Ser Lys Ser Asp Ile Ser Gly Ser Cys Gly Ile Ser Ala Gly Gly
                245                 250                 255

Tyr Lys Glu Cys Pro Pro Asn Asp Arg Met Cys Gly Lys Ile Ile Cys
            260                 265                 270

Lys Tyr Gln Ser Glu Asn Ile Leu Lys Leu Arg Ser Ala Thr Val Ile
        275                 280                 285

Tyr Ala Asn Ile Ser Gly His Val Cys Val Ser Leu Glu Tyr Pro Gln
    290                 295                 300

Gly His Asn Glu Ser Gln Lys Met Trp Val Arg Asp Gly Thr Val Cys
305                 310                 315                 320

Gly Ser Asn Lys Val Cys Gln Asn Gln Lys Cys Val Ala Asp Thr Phe
                325                 330                 335

Leu Gly Tyr Asp Cys Asn Leu Glu Lys Cys Asn His His Gly Val Cys
            340                 345                 350

Asn Asn Lys Lys Asn Cys His Cys Asp Pro Thr Tyr Leu Pro Pro Asp
        355                 360                 365

Cys Lys Arg Met Lys Asp Ser Tyr Pro Gly Gly Ser Ile Asp Ser Gly
    370                 375                 380

Asn Lys Glu Arg Ala Glu Pro Ile Pro Val Arg Pro Tyr Ile Ala Ser
385                 390                 395                 400

Arg Tyr Arg Ser Lys Ser Pro Arg Trp Pro Phe Phe Leu Ile Ile Pro
                405                 410                 415

Phe Tyr Val Val Ile Leu Val Leu Ile Gly Met Leu Val Lys Val Tyr
            420                 425                 430

Ser Gln Arg Met Lys Trp Arg Met Asp Asp Phe Ser Ser Glu Glu Gln
        435                 440                 445

Phe Glu Ser Glu Ser Glu Ser Lys Asp
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2553 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17..2221

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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TGAGGAGGAC CAGCGC ATG CGG CTC ATC TTG CTT CTA CTG AGT GGG CTG          49
                  Met Arg Leu Ile Leu Leu Leu Leu Ser Gly Leu
                    1               5                  10

AGT GAA CTT GGC GGC CTT AGC CAG TCC CAA ACA GAA GGC ACT CGT GAG          97
Ser Glu Leu Gly Gly Leu Ser Gln Ser Gln Thr Glu Gly Thr Arg Glu
            15                  20                  25

AAA TTA CAC GTG CAA GTC ACA GTG CCA GAG AAA ATC CGG TCC GTC ACA         145
Lys Leu His Val Gln Val Thr Val Pro Glu Lys Ile Arg Ser Val Thr
        30                  35                  40

AGC AAT GGC TAC GAA ACA CAG GTG ACC TAC AAT CTC AAA ATC GAA GGG         193
Ser Asn Gly Tyr Glu Thr Gln Val Thr Tyr Asn Leu Lys Ile Glu Gly
    45                  50                  55

AAA ACA TAC ACC TTG GAC CTA ATG CAA AAA CCG TTC TTG CCT CCC AAC         241
Lys Thr Tyr Thr Leu Asp Leu Met Gln Lys Pro Phe Leu Pro Pro Asn
60                  65                  70                  75

TTT AGA GTA TAC AGT TAT GAC AAC GCA GGA ATC ATG AGG TCT CTT GAG         289
Phe Arg Val Tyr Ser Tyr Asp Asn Ala Gly Ile Met Arg Ser Leu Glu
                80                  85                  90

CAG AAG TTT CAG AAT ATC TGC TAC TTC CAA GGA TAC ATT GAA GGT TAT         337
Gln Lys Phe Gln Asn Ile Cys Tyr Phe Gln Gly Tyr Ile Glu Gly Tyr
            95                  100                 105

CCA AAT TCT ATG GTG ATT GTT AGC ACA TGT ACT GGA CTG AGG GGT TTT         385
Pro Asn Ser Met Val Ile Val Ser Thr Cys Thr Gly Leu Arg Gly Phe
        110                 115                 120

CTC CAA TTT GGA AAC GTT AGC TAT GGA ATT GAA CCT CTG GAA TCT TCC         433
Leu Gln Phe Gly Asn Val Ser Tyr Gly Ile Glu Pro Leu Glu Ser Ser
    125                 130                 135
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AGT GGT TTT GAA CAC GTG ATC TAC CAA GTG GAA CCT GAG AAA GGA GGT      481
Ser Gly Phe Glu His Val Ile Tyr Gln Val Glu Pro Glu Lys Gly Gly
140                 145                 150                 155

GCA TTA CTC TAC GCC GAG AAG GAT ATC GAT TTA AGA GAC TCG CAG TAT      529
Ala Leu Leu Tyr Ala Glu Lys Asp Ile Asp Leu Arg Asp Ser Gln Tyr
                160                 165                 170

AAG ATA CGA AGT ATC AAG CCA CAG CGG ATC GTC TCT CAC TAT TTG GAA      577
Lys Ile Arg Ser Ile Lys Pro Gln Arg Ile Val Ser His Tyr Leu Glu
            175                 180                 185

ATA CAT ATT GTC GTT GAA AAG CAA ATG TTT GAG CAT ATC GGG GCT GAT      625
Ile His Ile Val Val Glu Lys Gln Met Phe Glu His Ile Gly Ala Asp
        190                 195                 200

ACA GCC ATT GTC ACT CAA AAG ATT TTC CAG TTG ATT GGA CTG GCA AAT      673
Thr Ala Ile Val Thr Gln Lys Ile Phe Gln Leu Ile Gly Leu Ala Asn
    205                 210                 215

GCT ATC TTT GCC CCC TTT AAT CTT ACA GTA ATT CTG TCT TCC CTG GAA      721
Ala Ile Phe Ala Pro Phe Asn Leu Thr Val Ile Leu Ser Ser Leu Glu
220                 225                 230                 235

TTT TGG ATG GAT GAA AAC AAA ATC TTG ACC ACA GGC GAT GCT AAC AAG      769
Phe Trp Met Asp Glu Asn Lys Ile Leu Thr Thr Gly Asp Ala Asn Lys
                240                 245                 250

TTG CTC TAC AGG TTC CTG AAG TGG AAA CAG TCG TAC CTT GTT CTG CGA      817
Leu Leu Tyr Arg Phe Leu Lys Trp Lys Gln Ser Tyr Leu Val Leu Arg
            255                 260                 265

CCA CAT GAT ATG GCG TTT TTA CTC GTC TAC AGG AAC ACT ACC GAT TAT      865
Pro His Asp Met Ala Phe Leu Leu Val Tyr Arg Asn Thr Thr Asp Tyr
        270                 275                 280

GTT GGC GCT ACC TAT CAA GGG AAG ATG TGT GAC AAG AAC TAT GCA GGA      913
Val Gly Ala Thr Tyr Gln Gly Lys Met Cys Asp Lys Asn Tyr Ala Gly
    285                 290                 295

GGA GTT GCT TTG CAC CCC AAA GCC GTA ACT CTG GAA TCA CTT GCA ATT      961
Gly Val Ala Leu His Pro Lys Ala Val Thr Leu Glu Ser Leu Ala Ile
300                 305                 310                 315

ATT TTA GTT CAG CTG CTG AGC CTC AGC ATG GGG CTA GCG TAT GAC GAC     1009
Ile Leu Val Gln Leu Leu Ser Leu Ser Met Gly Leu Ala Tyr Asp Asp
                320                 325                 330

GTG AAC AAG TGC CAG TGT GGC GTA CCT GTC TGC GTG ATG AAC CCG GAA     1057
Val Asn Lys Cys Gln Cys Gly Val Pro Val Cys Val Met Asn Pro Glu
            335                 340                 345

GCG CCT CAC TCC AGC GGT GTC CGG GCC TTC AGT AAC TGC AGC ATG GAG     1105
Ala Pro His Ser Ser Gly Val Arg Ala Phe Ser Asn Cys Ser Met Glu
        350                 355                 360
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GAC TTT TCC AAG TTT ATC ACA AGT CAA AGC TCC CAC TGT CTG CAG AAC      1153
Asp Phe Ser Lys Phe Ile Thr Ser Gln Ser Ser His Cys Leu Gln Asn
    365                 370                 375

CAG CCA ACG CTA CAG CCA TCT TAC AAG ATG GCG GTC TGT GGG AAT GGA      1201
Gln Pro Thr Leu Gln Pro Ser Tyr Lys Met Ala Val Cys Gly Asn Gly
380                 385                 390                 395

GAG GTG GAA GAA GAT GAA ATT TGC GAC TGT GGA AAG AAG GGC TGT GCA      1249
Glu Val Glu Glu Asp Glu Ile Cys Asp Cys Gly Lys Lys Gly Cys Ala
                400                 405                 410

GAA ATG CCC CCG CCA TGC TGT AAC CCC GAC ACC TGT AAG CTG TCA GAT      1297
Glu Met Pro Pro Pro Cys Cys Asn Pro Asp Thr Cys Lys Leu Ser Asp
            415                 420                 425

GGC TCC GAG TGC TCC AGC GGG ATA TGC TGC AAC TCG TGC AAG CTG AAG      1345
Gly Ser Glu Cys Ser Ser Gly Ile Cys Cys Asn Ser Cys Lys Leu Lys
        430                 435                 440

CGG AAA GGG GAG GTT TGC AGG CTT GCC CAA GAT GAG TGT GAT GTC ACA      1393
Arg Lys Gly Glu Val Cys Arg Leu Ala Gln Asp Glu Cys Asp Val Thr
    445                 450                 455

GAG TAC TGC AAC GGC ACA TCC GAA GTG TGT GAA GAC TTC TTT GTT CAA      1441
Glu Tyr Cys Asn Gly Thr Ser Glu Val Cys Glu Asp Phe Phe Val Gln
460                 465                 470                 475

AAC GGT CAC CCA TGT GAC AAT CGC AAG TGG ATC TGT ATT AAC GGC ACC      1489
Asn Gly His Pro Cys Asp Asn Arg Lys Trp Ile Cys Ile Asn Gly Thr
                480                 485                 490

TGT CAG AGT GGA GAA CAG CAG TGC CAG GAT CTA TTT GGC ATC GAT GCA      1537
Cys Gln Ser Gly Glu Gln Gln Cys Gln Asp Leu Phe Gly Ile Asp Ala
            495                 500                 505

GGC TTT GGT TCA AGT GAA TGT TTC TGG GAG CTG AAT TCC AAG AGC GAC      1585
Gly Phe Gly Ser Ser Glu Cys Phe Trp Glu Leu Asn Ser Lys Ser Asp
        510                 515                 520

ATA TCT GGG AGC TGT GGA ATC TCT GCT GGG GGA TAC AAG GAA TGC CCA      1633
Ile Ser Gly Ser Cys Gly Ile Ser Ala Gly Gly Tyr Lys Glu Cys Pro
    525                 530                 535

CCT AAT GAC CGG ATG TGT GGG AAA ATA ATA TGT AAA TAC CAA AGT GAA      1681
Pro Asn Asp Arg Met Cys Gly Lys Ile Ile Cys Lys Tyr Gln Ser Glu
540                 545                 550                 555

AAT ATA CTA AAA TTG AGG TCT GCC ACT GTT ATT TAT GCC AAT ATA AGC      1729
Asn Ile Leu Lys Leu Arg Ser Ala Thr Val Ile Tyr Ala Asn Ile Ser
                560                 565                 570

GGG CAT GTC TGC GTT TCC CTG GAA TAT CCC CAA GGT CAT AAT GAG AGC      1777
Gly His Val Cys Val Ser Leu Glu Tyr Pro Gln Gly His Asn Glu Ser
            575                 580                 585
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CAG AAG ATG TGG GTG AGA GAT GGA ACC GTC TGC GGG TCA AAT AAG GTT      1825
Gln Lys Met Trp Val Arg Asp Gly Thr Val Cys Gly Ser Asn Lys Val
        590                 595                 600

TGC CAG AAT CAA AAA TGT GTA GCA GAC ACT TTC TTG GGC TAT GAT TGC      1873
Cys Gln Asn Gln Lys Cys Val Ala Asp Thr Phe Leu Gly Tyr Asp Cys
    605                 610                 615

AAC CTG GAA AAA TGC AAC CAC CAT GGT GTA TGT AAT AAC AAG AAG AAC      1921
Asn Leu Glu Lys Cys Asn His His Gly Val Cys Asn Asn Lys Lys Asn
620                 625                 630                 635

TGC CAC TGT GAC CCC ACA TAC TTA CCT CCA GAT TGT AAA AGA ATG AAA      1969
Cys His Cys Asp Pro Thr Tyr Leu Pro Pro Asp Cys Lys Arg Met Lys
                640                 645                 650

GAT TCA TAT CCT GGC GGG AGC ATT GAT AGT GGC AAC AAG GAA AGG GCT      2017
Asp Ser Tyr Pro Gly Gly Ser Ile Asp Ser Gly Asn Lys Glu Arg Ala
            655                 660                 665

GAA CCC ATC CCT GTA CGG CCC TAC ATT GCA AGT CGT TAC CGC TCC AAG      2065
Glu Pro Ile Pro Val Arg Pro Tyr Ile Ala Ser Arg Tyr Arg Ser Lys
        670                 675                 680

TCT CCA CGG TGG CCA TTT TTC TTG ATC ATC CCT TTC TAC GTT GTG ATC      2113
Ser Pro Arg Trp Pro Phe Phe Leu Ile Ile Pro Phe Tyr Val Val Ile
    685                 690                 695

CTT GTC CTG ATT GGG ATG CTG GTA AAA GTC TAT TCC CAA AGG ATG AAA      2161
Leu Val Leu Ile Gly Met Leu Val Lys Val Tyr Ser Gln Arg Met Lys
700                 705                 710                 715

TGG AGA ATG GAT GAC TTC TCA AGC GAA GAG CAA TTT GAA AGT GAA AGT      2209
Trp Arg Met Asp Asp Phe Ser Ser Glu Glu Gln Phe Glu Ser Glu Ser
                720                 725                 730

GAA TCC AAA GAC TAGTCTGGAC AGATTCCACA ATGTCACAAG TAATTCTCTT          2261
Glu Ser Lys Asp
            735

CAGTGGACAG AAAAAAAAGT GGAAAAGAAA AGCCTATGCA TTATCTTGCC TGAAAGTCAA   2321

GCCTGCATAT CGTGGTCTCC ATCAGGCCAG AAATCATATC TCTCCATTAC ACATGTATGA   2381

TACATATGTG TGTATATTAT TCCATAAATG ATTTACTTGT AAGAAATGAA TGATTATGAA   2441

TTTCATATTA TACTTTGATA TTTTACCCTA TTTCTGGTAG TCGGTAGTCA TCAATTGTAT   2501

TTTCTAGTAG GTACATTATA GAAAAGGCTA TAAGAAAATA AATGTGGTAC CA           2553
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 735 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Arg Leu Ile Leu Leu Leu Leu Ser Gly Leu Ser Glu Leu Gly Gly
 1               5                  10                  15

Leu Ser Gln Ser Gln Thr Glu Gly Thr Arg Glu Lys Leu His Val Gln
            20                  25                  30

Val Thr Val Pro Glu Lys Ile Arg Ser Val Thr Ser Asn Gly Tyr Glu
        35                  40                  45

Thr Gln Val Thr Tyr Asn Leu Lys Ile Glu Gly Lys Thr Tyr Thr Leu
    50                  55                  60

Asp Leu Met Gln Lys Pro Phe Leu Pro Pro Asn Phe Arg Val Tyr Ser
65                  70                  75                  80

Tyr Asp Asn Ala Gly Ile Met Arg Ser Leu Glu Gln Lys Phe Gln Asn
                85                  90                  95

Ile Cys Tyr Phe Gln Gly Tyr Ile Glu Gly Tyr Pro Asn Ser Met Val
            100                 105                 110

Ile Val Ser Thr Cys Thr Gly Leu Arg Gly Phe Leu Gln Phe Gly Asn
        115                 120                 125

Val Ser Tyr Gly Ile Glu Pro Leu Glu Ser Ser Ser Gly Phe Glu His
    130                 135                 140

Val Ile Tyr Gln Val Glu Pro Glu Lys Gly Gly Ala Leu Leu Tyr Ala
145                 150                 155                 160

Glu Lys Asp Ile Asp Leu Arg Asp Ser Gln Tyr Lys Ile Arg Ser Ile
                165                 170                 175

Lys Pro Gln Arg Ile Val Ser His Tyr Leu Glu Ile His Ile Val Val
            180                 185                 190

Glu Lys Gln Met Phe Glu His Ile Gly Ala Asp Thr Ala Ile Val Thr
        195                 200                 205

Gln Lys Ile Phe Gln Leu Ile Gly Leu Ala Asn Ala Ile Phe Ala Pro
    210                 215                 220

Phe Asn Leu Thr Val Ile Leu Ser Ser Leu Glu Phe Trp Met Asp Glu
225                 230                 235                 240

Asn Lys Ile Leu Thr Thr Gly Asp Ala Asn Lys Leu Leu Tyr Arg Phe
                245                 250                 255

Leu Lys Trp Lys Gln Ser Tyr Leu Val Leu Arg Pro His Asp Met Ala
            260                 265                 270
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Phe Leu Leu Val Tyr Arg Asn Thr Thr Asp Tyr Val Gly Ala Thr Tyr
        275                 280                 285

Gln Gly Lys Met Cys Asp Lys Asn Tyr Ala Gly Gly Val Ala Leu His
    290                 295                 300

Pro Lys Ala Val Thr Leu Glu Ser Leu Ala Ile Ile Leu Val Gln Leu
305                 310                 315                 320

Leu Ser Leu Ser Met Gly Leu Ala Tyr Asp Asp Val Asn Lys Cys Gln
                325                 330                 335

Cys Gly Val Pro Val Cys Val Met Asn Pro Glu Ala Pro His Ser Ser
            340                 345                 350

Gly Val Arg Ala Phe Ser Asn Cys Ser Met Glu Asp Phe Ser Lys Phe
        355                 360                 365

Ile Thr Ser Gln Ser Ser His Cys Leu Gln Asn Gln Pro Thr Leu Gln
    370                 375                 380

Pro Ser Tyr Lys Met Ala Val Cys Gly Asn Gly Glu Val Glu Glu Asp
385                 390                 395                 400

Glu Ile Cys Asp Cys Gly Lys Lys Gly Cys Ala Glu Met Pro Pro Pro
                405                 410                 415

Cys Cys Asn Pro Asp Thr Cys Lys Leu Ser Asp Gly Ser Glu Cys Ser
            420                 425                 430

Ser Gly Ile Cys Cys Asn Ser Cys Lys Leu Lys Arg Lys Gly Glu Val
        435                 440                 445

Cys Arg Leu Ala Gln Asp Glu Cys Asp Val Thr Glu Tyr Cys Asn Gly
    450                 455                 460

Thr Ser Glu Val Cys Glu Asp Phe Phe Val Gln Asn Gly His Pro Cys
465                 470                 475                 480

Asp Asn Arg Lys Trp Ile Cys Ile Asn Gly Thr Cys Gln Ser Gly Glu
                485                 490                 495

Gln Gln Cys Gln Asp Leu Phe Gly Ile Asp Ala Gly Phe Gly Ser Ser
            500                 505                 510

Glu Cys Phe Trp Glu Leu Asn Ser Lys Ser Asp Ile Ser Gly Ser Cys
        515                 520                 525

Gly Ile Ser Ala Gly Gly Tyr Lys Glu Cys Pro Pro Asn Asp Arg Met
    530                 535                 540

Cys Gly Lys Ile Ile Cys Lys Tyr Gln Ser Glu Asn Ile Leu Lys Leu
545                 550                 555                 560
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Arg Ser Ala Thr Val Ile Tyr Ala Asn Ile Ser Gly His Val Cys Val
                565                 570                 575

Ser Leu Glu Tyr Pro Gln Gly His Asn Glu Ser Gln Lys Met Trp Val
            580                 585                 590

Arg Asp Gly Thr Val Cys Gly Ser Asn Lys Val Cys Gln Asn Gln Lys
        595                 600                 605

Cys Val Ala Asp Thr Phe Leu Gly Tyr Asp Cys Asn Leu Glu Lys Cys
    610                 615                 620

Asn His His Gly Val Cys Asn Asn Lys Lys Asn Cys His Cys Asp Pro
625                 630                 635                 640

Thr Tyr Leu Pro Pro Asp Cys Lys Arg Met Lys Asp Ser Tyr Pro Gly
                645                 650                 655

Gly Ser Ile Asp Ser Gly Asn Lys Glu Arg Ala Glu Pro Ile Pro Val
            660                 665                 670

Arg Pro Tyr Ile Ala Ser Arg Tyr Arg Ser Lys Ser Pro Arg Trp Pro
        675                 680                 685

Phe Phe Leu Ile Ile Pro Phe Tyr Val Val Ile Leu Val Leu Ile Gly
    690                 695                 700

Met Leu Val Lys Val Tyr Ser Gln Arg Met Lys Trp Arg Met Asp Asp
705                 710                 715                 720

Phe Ser Ser Glu Glu Gln Phe Glu Ser Glu Ser Glu Ser Lys Asp
                725                 730                 735
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 72..2273

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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CATCTCGCAC TTCCAACTGC CCTGTAACCA CCAACTGCCC TTATTCCGGC TGGGACCCAG      60

GACTTCAAGC C ATG TGG GTC TTG TTT CTG CTC AGC GGG CTC GGC GGG CTG      110
             Met Trp Val Leu Phe Leu Leu Ser Gly Leu Gly Gly Leu
                         740                 745
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CGG ATG GAC AGT AAT TTT GAT AGT TTA CCT GTG CAA ATT ACA GTT CCG        158
Arg Met Asp Ser Asn Phe Asp Ser Leu Pro Val Gln Ile Thr Val Pro
    750                 755                 760

GAG AAA ATA CGG TCA ATA ATA AAG GAA GGA ATT GAA TCG CAG GCA TCC        206
Glu Lys Ile Arg Ser Ile Ile Lys Glu Gly Ile Glu Ser Gln Ala Ser
765                 770                 775                 780

TAC AAA ATT GTA ATT GAA GGG AAA CCA TAT ACT GTG AAT TTA ATG CAA        254
Tyr Lys Ile Val Ile Glu Gly Lys Pro Tyr Thr Val Asn Leu Met Gln
                785                 790                 795

AAA AAC TTT TTA CCC CAT AAT TTT AGA GTT TAC AGT TAT AGT GGC ACA        302
Lys Asn Phe Leu Pro His Asn Phe Arg Val Tyr Ser Tyr Ser Gly Thr
            800                 805                 810

GGA ATT ATG AAA CCA CTT GAC CAA GAT TTT CAG AAT TTC TGC CAC TAC        350
Gly Ile Met Lys Pro Leu Asp Gln Asp Phe Gln Asn Phe Cys His Tyr
        815                 820                 825

CAA GGG TAT ATT GAA GGT TAT CCA AAA TCT GTG GTG ATG GTT AGC ACA        398
Gln Gly Tyr Ile Glu Gly Tyr Pro Lys Ser Val Val Met Val Ser Thr
    830                 835                 840

TGT ACT GGA CTC AGG GGC GTA CTA CAG TTT GAA AAT GTT AGT TAT GGA        446
Cys Thr Gly Leu Arg Gly Val Leu Gln Phe Glu Asn Val Ser Tyr Gly
845                 850                 855                 860

ATA GAA CCC CTG GAG TCT TCA GTT GGC TTT GAA CAT GTA ATT TAC CAA        494
Ile Glu Pro Leu Glu Ser Ser Val Gly Phe Glu His Val Ile Tyr Gln
                865                 870                 875

GTA AAA CAT AAG AAA GCA GAT GTT TCC TTA TAT AAT GAG AAG GAT ATT        542
Val Lys His Lys Lys Ala Asp Val Ser Leu Tyr Asn Glu Lys Asp Ile
            880                 885                 890

GAA TCA AGA GAT CTG TCC TTT AAA TTA CAA AGC GCA GAG CCA CAG CAA        590
Glu Ser Arg Asp Leu Ser Phe Lys Leu Gln Ser Ala Glu Pro Gln Gln
        895                 900                 905

GAT TTT GCA AAG TAT ATA GAA ATG CAT GTT ATA GTT GAA AAA CAA TTG        638
Asp Phe Ala Lys Tyr Ile Glu Met His Val Ile Val Glu Lys Gln Leu
    910                 915                 920

TAT AAT CAT ATG GGG TCT GAT ACA ACT GTT GTC GCT CAA AAA GTT TTC        686
Tyr Asn His Met Gly Ser Asp Thr Thr Val Val Ala Gln Lys Val Phe
925                 930                 935                 940

CAG TTG ATT GGA TTG ACG AAT GCT ATT TTT GTT TCA TTT AAT ATT ACA        734
Gln Leu Ile Gly Leu Thr Asn Ala Ile Phe Val Ser Phe Asn Ile Thr
                945                 950                 955

ATT ATT CTG TCT TCA TTG GAG CTT TGG ATA GAT GAA AAT AAA ATT GCA        782
Ile Ile Leu Ser Ser Leu Glu Leu Trp Ile Asp Glu Asn Lys Ile Ala
            960                 965                 970
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ACC ACT GGA GAA GCT AAT GAG TTA TTA CAC ACA TTT TTA AGA TGG AAA      830
Thr Thr Gly Glu Ala Asn Glu Leu Leu His Thr Phe Leu Arg Trp Lys
      975                 980                 985

ACA TCT TAT CTT GTT TTA CGT CCT CAT GAT GTG GCA TTT TTA CTT GTT      878
Thr Ser Tyr Leu Val Leu Arg Pro His Asp Val Ala Phe Leu Leu Val
    990                 995                1000

TAC AGA GAA AAG TCA AAT TAT GTT GGT GCA ACC TTT CAA GGG AAG ATG      926
Tyr Arg Glu Lys Ser Asn Tyr Val Gly Ala Thr Phe Gln Gly Lys Met
1005                1010                1015                1020

TGT GAT GCA AAC TAT GCA GGA GGT GTT GTT CTG CAC CCC AGA ACC ATA      974
Cys Asp Ala Asn Tyr Ala Gly Gly Val Val Leu His Pro Arg Thr Ile
                1025                1030                1035

AGT CTG GAA TCA CTT GCA GTT ATT TTA GCT CAA TTA TTG AGC CTT AGT     1022
Ser Leu Glu Ser Leu Ala Val Ile Leu Ala Gln Leu Leu Ser Leu Ser
            1040                1045                1050

ATG GGG ATC ACT TAT GAT GAC ATT AAC AAA TGC CAG TGC TCA GGA GCT     1070
Met Gly Ile Thr Tyr Asp Asp Ile Asn Lys Cys Gln Cys Ser Gly Ala
        1055                1060                1065

GTC TGC ATT ATG AAT CCA GAA GCA ATT CAT TTC AGT GGT GTG AAG ATC     1118
Val Cys Ile Met Asn Pro Glu Ala Ile His Phe Ser Gly Val Lys Ile
    1070                1075                1080

TTT AGT AAC TGC AGC TTC GAA GAC TTT GCA CAT TTT ATT TCA AAG CAG     1166
Phe Ser Asn Cys Ser Phe Glu Asp Phe Ala His Phe Ile Ser Lys Gln
1085                1090                1095                1100

AAG TCC CAG TGT CTT CAC AAT CAG CCT CGC TTA GAT CCT TTT TTC AAA     1214
Lys Ser Gln Cys Leu His Asn Gln Pro Arg Leu Asp Pro Phe Phe Lys
                1105                1110                1115

CAG CAA GCA GTG TGT GGT AAT GCA AAG CTG GAA GCA GGA GAG GAG TGT     1262
Gln Gln Ala Val Cys Gly Asn Ala Lys Leu Glu Ala Gly Glu Glu Cys
            1120                1125                1130

GAC TGT GGG ACT GAA CAG GAT TGT GCC CTT ATT GGA GAA ACA TGC TGT     1310
Asp Cys Gly Thr Glu Gln Asp Cys Ala Leu Ile Gly Glu Thr Cys Cys
        1135                1140                1145

GAT ATT GCC ACA TGT AGA TTT AAA GCC GGT TCA AAC TGT GCT GAA GGA     1358
Asp Ile Ala Thr Cys Arg Phe Lys Ala Gly Ser Asn Cys Ala Glu Gly
    1150                1155                1160

CCA TGC TGC GAA AAC TGT CTA TTT ATG TCA AAA GAA AGA ATG TGT AGG     1406
Pro Cys Cys Glu Asn Cys Leu Phe Met Ser Lys Glu Arg Met Cys Arg
1165                1170                1175                1180

CCT TCC TTT GAA GAA TGC GAC CTC CCT GAA TAT TGC AAT GGA TCA TCT     1454
Pro Ser Phe Glu Glu Cys Asp Leu Pro Glu Tyr Cys Asn Gly Ser Ser
                1185                1190                1195
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GCA TCA TGC CCA GAA AAC CAC TAT GTT CAG ACT GGG CAT CCG TGT GGA      1502
Ala Ser Cys Pro Glu Asn His Tyr Val Gln Thr Gly His Pro Cys Gly
            1200                1205                1210

CTG AAT CAA TGG ATC TGT ATA GAT GGA GTT TGT ATG AGT GGG GAT AAA      1550
Leu Asn Gln Trp Ile Cys Ile Asp Gly Val Cys Met Ser Gly Asp Lys
        1215                1220                1225

CAA TGT ACA GAC ACA TTT GGC AAA GAA GTA GAG TTT GGC CCT TCA GAA      1598
Gln Cys Thr Asp Thr Phe Gly Lys Glu Val Glu Phe Gly Pro Ser Glu
    1230                1235                1240

TGT TAT TCT CAC CTT AAT TCA AAG ACT GAT GTA TCT GGA AAC TGT GGT      1646
Cys Tyr Ser His Leu Asn Ser Lys Thr Asp Val Ser Gly Asn Cys Gly
1245                1250                1255                1260

ATA AGT GAT TCA GGA TAC ACA CAG TGT GAA GCT GAC AAT CTG CAG TGC      1694
Ile Ser Asp Ser Gly Tyr Thr Gln Cys Glu Ala Asp Asn Leu Gln Cys
                1265                1270                1275

GGA AAA TTA ATA TGT AAA TAT GTA GGT AAA TTT TTA TTA CAA ATT CCA      1742
Gly Lys Leu Ile Cys Lys Tyr Val Gly Lys Phe Leu Leu Gln Ile Pro
            1280                1285                1290

AGA GCC ACT ATT ATT TAT GCC AAC ATA AGT GGA CAT CTC TGC ATT GCT      1790
Arg Ala Thr Ile Ile Tyr Ala Asn Ile Ser Gly His Leu Cys Ile Ala
        1295                1300                1305

GTG GAA TTT GCC AGT GAT CAT GCA GAC AGC CAA AAG ATG TGG ATA AAA      1838
Val Glu Phe Ala Ser Asp His Ala Asp Ser Gln Lys Met Trp Ile Lys
    1310                1315                1320

GAT GGA ACT TCT TGT GGT TCA AAT AAG GTT TGC AGG AAT CAA AGA TGT      1886
Asp Gly Thr Ser Cys Gly Ser Asn Lys Val Cys Arg Asn Gln Arg Cys
1325                1330                1335                1340

GTG AGT TCT TCA TAC TTG GGT TAT GAT TGT ACT ACT GAC AAA TGC AAT      1934
Val Ser Ser Ser Tyr Leu Gly Tyr Asp Cys Thr Thr Asp Lys Cys Asn
                1345                1350                1355

GAT AGA GGT GTA TGC AAT AAC AAA AAG CAC TGT CAC TGT AGT GCT TCA      1982
Asp Arg Gly Val Cys Asn Asn Lys Lys His Cys His Cys Ser Ala Ser
            1360                1365                1370

TAT TTA CCT CCA GAT TGC TCA GTT CAA TCA GAT CTA TGG CCT GGT GGG      2030
Tyr Leu Pro Pro Asp Cys Ser Val Gln Ser Asp Leu Trp Pro Gly Gly
        1375                1380                1385

AGT ATT GAC AGT GGC AAT TTT CCA CCT GTA GCT ATA CCA GCC AGA CTC      2078
Ser Ile Asp Ser Gly Asn Phe Pro Pro Val Ala Ile Pro Ala Arg Leu
    1390                1395                1400

CCT GAA AGG CGC TAC ATT GAG AAC ATT TAC CAT TCC AAA CCA ATG AGA      2126
Pro Glu Arg Arg Tyr Ile Glu Asn Ile Tyr His Ser Lys Pro Met Arg
1405                1410                1415                1420
```

```
TGG CCA TTT TTC TTA TTC ATT CCT TTC TTT ATT ATT TTC TGT GTA CTG      2174
Trp Pro Phe Phe Leu Phe Ile Pro Phe Phe Ile Ile Phe Cys Val Leu
                1425                1430                1435

ATT GCT ATA ATG GTG AAA GTT AAT TTC CAA AGG AAA AAA TGG AGA ACT      2222
Ile Ala Ile Met Val Lys Val Asn Phe Gln Arg Lys Lys Trp Arg Thr
            1440                1445                1450

GAG GAC TAT TCA AGC GAT GAG CAA CCT GAA AGT GAG AGT GAA CCT AAA      2270
Glu Asp Tyr Ser Ser Asp Glu Gln Pro Glu Ser Glu Ser Glu Pro Lys
        1455                1460                1465

GGG TAGTCTGGAC AACAGAGATG CCATGATATC ACTTCTTCTA GAGTAATTAT          2323
Gly
```

```
CTGTGATGGA TGGACACAAA AAAATGGAAA GAAAAGAATG TACATTACCT GGTTTCCTGG   2383

GATTCAAACC TGCATATTGT GATTTTAATT TGACCAGAAA ATATGATATA TATGTATAAT   2443

TTCACAGATA ATTTACTTAT TTAAAAATGC ATGATAATGA GTTTTACATT ACAAATTTCT   2503

GTTTTTTTAA AGTTATCTTA CGCTATTTCT GTTGGTTAGT AGACACTAAT TCTGTCAGTA   2563

GGGGCATGGT ATAAGGAAAT ATCATAATGT AATGAGGTGG TACTATGATT AAAAGCCACT   2623

GTTACATTTC AAAAAAAAAA AAAAAAA                                       2650
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 734 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Trp Val Leu Phe Leu Leu Ser Gly Leu Gly Gly Leu Arg Met Asp
 1               5                  10                  15

Ser Asn Phe Asp Ser Leu Pro Val Gln Ile Thr Val Pro Glu Lys Ile
            20                  25                  30

Arg Ser Ile Ile Lys Glu Gly Ile Glu Ser Gln Ala Ser Tyr Lys Ile
        35                  40                  45

Val Ile Glu Gly Lys Pro Tyr Thr Val Asn Leu Met Gln Lys Asn Phe
    50                  55                  60

Leu Pro His Asn Phe Arg Val Tyr Ser Tyr Ser Gly Thr Gly Ile Met
65                  70                  75                  80
```

```
Lys Pro Leu Asp Gln Asp Phe Gln Asn Phe Cys His Tyr Gln Gly Tyr
                85                  90                  95

Ile Glu Gly Tyr Pro Lys Ser Val Val Met Val Ser Thr Cys Thr Gly
            100                 105                 110

Leu Arg Gly Val Leu Gln Phe Glu Asn Val Ser Tyr Gly Ile Glu Pro
        115                 120                 125

Leu Glu Ser Ser Val Gly Phe Glu His Val Ile Tyr Gln Val Lys His
    130                 135                 140

Lys Lys Ala Asp Val Ser Leu Tyr Asn Glu Lys Asp Ile Glu Ser Arg
145                 150                 155                 160

Asp Leu Ser Phe Lys Leu Gln Ser Ala Glu Pro Gln Gln Asp Phe Ala
                165                 170                 175

Lys Tyr Ile Glu Met His Val Ile Val Glu Lys Gln Leu Tyr Asn His
            180                 185                 190

Met Gly Ser Asp Thr Thr Val Val Ala Gln Lys Val Phe Gln Leu Ile
        195                 200                 205

Gly Leu Thr Asn Ala Ile Phe Val Ser Phe Asn Ile Thr Ile Ile Leu
    210                 215                 220

Ser Ser Leu Glu Leu Trp Ile Asp Glu Asn Lys Ile Ala Thr Thr Gly
225                 230                 235                 240

Glu Ala Asn Glu Leu Leu His Thr Phe Leu Arg Trp Lys Thr Ser Tyr
                245                 250                 255

Leu Val Leu Arg Pro His Asp Val Ala Phe Leu Leu Val Tyr Arg Glu
            260                 265                 270

Lys Ser Asn Tyr Val Gly Ala Thr Phe Gln Gly Lys Met Cys Asp Ala
        275                 280                 285

Asn Tyr Ala Gly Gly Val Val Leu His Pro Arg Thr Ile Ser Leu Glu
    290                 295                 300

Ser Leu Ala Val Ile Leu Ala Gln Leu Leu Ser Leu Ser Met Gly Ile
305                 310                 315                 320

Thr Tyr Asp Asp Ile Asn Lys Cys Gln Cys Ser Gly Ala Val Cys Ile
                325                 330                 335

Met Asn Pro Glu Ala Ile His Phe Ser Gly Val Lys Ile Phe Ser Asn
            340                 345                 350

Cys Ser Phe Glu Asp Phe Ala His Phe Ile Ser Lys Gln Lys Ser Gln
        355                 360                 365

Cys Leu His Asn Gln Pro Arg Leu Asp Pro Phe Phe Lys Gln Gln Ala
    370                 375                 380
```

```
Val Cys Gly Asn Ala Lys Leu Glu Ala Gly Glu Glu Cys Asp Cys Gly
385                 390                 395                 400

Thr Glu Gln Asp Cys Ala Leu Ile Gly Glu Thr Cys Cys Asp Ile Ala
                405                 410                 415

Thr Cys Arg Phe Lys Ala Gly Ser Asn Cys Ala Glu Gly Pro Cys Cys
            420                 425                 430

Glu Asn Cys Leu Phe Met Ser Lys Glu Arg Met Cys Arg Pro Ser Phe
        435                 440                 445

Glu Glu Cys Asp Leu Pro Glu Tyr Cys Asn Gly Ser Ser Ala Ser Cys
    450                 455                 460

Pro Glu Asn His Tyr Val Gln Thr Gly His Pro Cys Gly Leu Asn Gln
465                 470                 475                 480

Trp Ile Cys Ile Asp Gly Val Cys Met Ser Gly Asp Lys Gln Cys Thr
                485                 490                 495

Asp Thr Phe Gly Lys Glu Val Glu Phe Gly Pro Ser Glu Cys Tyr Ser
            500                 505                 510

His Leu Asn Ser Lys Thr Asp Val Ser Gly Asn Cys Gly Ile Ser Asp
        515                 520                 525

Ser Gly Tyr Thr Gln Cys Glu Ala Asp Asn Leu Gln Cys Gly Lys Leu
    530                 535                 540

Ile Cys Lys Tyr Val Gly Lys Phe Leu Leu Gln Ile Pro Arg Ala Thr
545                 550                 555                 560

Ile Ile Tyr Ala Asn Ile Ser Gly His Leu Cys Ile Ala Val Glu Phe
                565                 570                 575

Ala Ser Asp His Ala Asp Ser Gln Lys Met Trp Ile Lys Asp Gly Thr
            580                 585                 590

Ser Cys Gly Ser Asn Lys Val Cys Arg Asn Gln Arg Cys Val Ser Ser
        595                 600                 605

Ser Tyr Leu Gly Tyr Asp Cys Thr Thr Asp Lys Cys Asn Asp Arg Gly
    610                 615                 620

Val Cys Asn Asn Lys Lys His Cys His Cys Ser Ala Ser Tyr Leu Pro
625                 630                 635                 640

Pro Asp Cys Ser Val Gln Ser Asp Leu Trp Pro Gly Gly Ser Ile Asp
                645                 650                 655

Ser Gly Asn Phe Pro Pro Val Ala Ile Pro Ala Arg Leu Pro Glu Arg
            660                 665                 670
```

```
Arg Tyr Ile Glu Asn Ile Tyr His Ser Lys Pro Met Arg Trp Pro Phe
        675                 680                 685

Phe Leu Phe Ile Pro Phe Phe Ile Ile Phe Cys Val Leu Ile Ala Ile
    690                 695                 700

Met Val Lys Val Asn Phe Gln Arg Lys Lys Trp Arg Thr Glu Asp Tyr
705                 710                 715                 720

Ser Ser Asp Glu Gln Pro Glu Ser Glu Ser Glu Pro Lys Gly
                725                 730
```

WHAT IS CLAIMED IS:

1. A sperm protein in substantially pure form selected from a human PH30 beta chain protein, a mouse PH30 beta chain protein or an amino acid sequence substantially homologous to either the human or mouse PH30 beta chain protein.

2. The sperm protein of Claim 1, having an integrin binding sequence which is not TDE.

3. The sperm protein of Claim 2, wherein the integrin binding sequence is selected from FEE or QDE.

4. The sperm protein of Claim 1 which is the human PH30 beta chain protein.

5. The sperm protein of Claim 4, having an integrin binding sequence which is FEE.

6. A DNA sequence which encodes the sperm protein of Claim 1 or a portion of the sperm protein sufficient to constitute at least one epitope.

7. The DNA sequence of Claim 6, wherein the epitope is on the native protein.

8. The DNA sequence of Claim 6 which encodes all or a portion of human PH30 beta chain protein.

9. The DNA sequence of Claim 8, wherein the DNA encoding all or a portion of the human PH30 beta protein is

characterized by the ability to hybridize, under standard conditions, to the DNA sequence shown in SEQ ID NO: 1.

10. A contraceptive composition comprising a therapeutically effective amount of the protein of Claim 1, or a polypeptide having the substantially same amino acid sequence as a segment of the protein provided that the polypeptide is sufficient to constitute at least one epitope, and a pharmaceutically acceptable carrier.

11. The contraceptive composition of Claim 10, wherein the epitope is on the native protein.

12. The contraceptive composition of Claim 10, wherein the protein is the human PH30 beta chain protein.

13. The contraceptive composition of Claim 10, wherein the protein is produced by expressing the gene encoding an immunogenic epitope of the sperm protein in a recombinant DNA expression vector.

14. A vector comprising an inserted DNA sequence encoding for the protein of Claim 1.

15. The vector of Claim 14, wherein the inserted DNA sequence is characterized by the ability to hybridize, under standard conditions, to a DNA sequence selected from the DNA sequences of SEQ ID NO: 1 or SEQ ID NO: 3.

16. A host that is compatible with and contains the vector of Claim 14.

17. A method of producing a human or mouse PH30 beta chain sperm protein, comprising the steps of culturing cells containing the DNA of Claim 6 and recovering the sperm protein from the cell culture.

18. The method of Claim 17, wherein the DNA encoding all or a portion of the PH30 beta chain protein is characterized by the ability to hybridize, under standard conditions, to a DNA sequence selected from the DNA sequences of SEQ ID NO: 1 or SEQ ID NO: 3.

19. A method of contraception in a human or mouse subject in need thereof, comprising administering to the subject an amount of the sperm protein of Claim 1 which is effective for the stimulation of antibodies which bind to the sperm protein in vivo.

20. The method of Claim 19, wherein the sperm protein has an integrin binding sequence which is not TDE.

21. A PH30 beta chain protein made by the process of Claim 17.

22. A DNA sequence as shown in Seq. ID No. 1 encoding human PH30 beta chain protein.

23. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of human or mouse PH30 beta to allow the possession of the biological property of initiating sperm-egg binding or promoting sperm-egg fusion.

24. The DNA sequence of Claim 23 wherein the amino acid sequence contains an integrin binding sequence which is not TDE.

```
                10                  30                  50
                .   .   .   .   .   .   .   .   .   .   .   .

1   GGCCAAGATTTTCAGAATTTCTGCCACTACCAAGGGTATATTGAAGGTTATCCAAAATCT  60

    GlyGlnAspPheGlnAsnPheCysHisTyrGlnGlyTyrIleGluGlyTyrProLysSer

                70                  90                  110
                .   .   .   .   .   .   .   .   .   .   .   .

61  GTGGTGATGGTTAGCACATGTACTGGACTCAGGGGCGTACTACAGTTTGAAAATGTTAGT  120

    ValValMetValSerThrCysThrGlyLeuArgGlyValLeuGlnPheGluAsnValSer

                130                 150                 170
                .   .   .   .   .   .   .   .   .   .   .   .

121 TATGGAATAGAACCCCTGGAGTCTTCAGTTGGCTTTGAACATGTAATTTACCAAGTAAAA  180

    TyrGlyIleGluProLeuGluSerSerValGlyPheGluHisValIleTyrGlnValLys

                190                 210                 230
                .   .   .   .   .   .   .   .   .   .   .   .

181 CATAAGAAAGCAGATGTTTCCTTATATAATGAGAAGGATATTGAATCAAGAGATCTGTCC  240

    HisLysLysAlaAspValSerLeuTyrAsnGluLysAspIleGluSerArgAspLeuSer

                250                 270                 290
                .   .   .   .   .   .   .   .   .   .   .   .

241 TTTAAATTACAAAGCGCAGAGCCACAGCAAGATTTTGCAAAGTATATAGAAATGCATGTT  300

    PheLysLeuGlnSerAlaGluProGlnGlnAspPheAlaLysTyrIleGluMetHisVal

                310                 330                 350
                .   .   .   .   .   .   .   .   .   .   .   .

301 ATAGTTGAAAAACAATTGTATAATCATATGGGGTCTGATACAACTGTTGTCGCTCAAAAA  360

    IleValGluLysGlnLeuTyrAsnHisMetGlySerAspThrThrValValAlaGlnLys
```

FIG.1A

```
                370                 390                 410
                 .   .   .   .   .   .   .   .   .   .   .   .
361 GTTTTCCAGTTGATTGGATTGACGAATGCTATTTTTGTTTCATTTAATATTACAATTATT 420
    ValPheGlnLeuIleGlyLeuThrAsnAlaIlePheValSerPheAsnIleThrIleIle

                430                 450                 470
                 .   .   .   .   .   .   .   .   .   .   .   .
421 CTGTCTTCATTGGAGCTTTGGATAGATGAAAATAAAATTGCAACCACTGGAGAAGCTAAT 480
    LeuSerSerLeuGluLeuTrpIleAspGluAsnLysIleAlaThrThrGlyGluAlaAsn

                490                 510                 530
                 .   .   .   .   .   .   .   .   .   .   .   .
481 GAGTTATTACACACATTTTTAAGATGGAAAACATCTTATCTTGTTTTACGTCCTCATGAT 540
    GluLeuLeuHisThrPheLeuArgTrpLysThrSerTyrLeuValLeuArgProHisAsp

                550                 570                 590
                 .   .   .   .   .   .   .   .   .   .   .   .
541 GTGGCATTTTTACTTGTTTACAGAGAAAAGTCAAATTATGTTGGTGCAACCTTTCAAGGG 600
    ValAlaPheLeuLeuValTyrArgGluLysSerAsnTyrValGlyAlaThrPheGlnGly

                610                 630                 650
                 .   .   .   .   .   .   .   .   .   .   .   .
601 AAGATGTGTGATGCAAACTATGCAGGAGGTGTTGTTCTGCACCCCAGAACCATAAGTCTG 660
    LysMetCysAspAlaAsnTyrAlaGlyGlyValValLeuHisProArgThrIleSerLeu

                670                 690                 710
                 .   .   .   .   .   .   .   .   .   .   .   .
661 GAATCACTTGCAGTTATTTTAGCTCAATTATTGAGCCTTAGTATGGGGATCACTTATGAT 720
    GluSerLeuAlaValIleLeuAlaGlnLeuLeuSerLeuSerMetGlyIleThrTyrAsp
```

FIG.1B

730 750 770

721 GACATTAACAAATGCCAGTGCTCAGGAGCTGTCTGCATTATGAATCCAGAAGCAATTCAT 780

AspIleAsnLysCysGlnCysSerGlyAlaValCysIleMetAsnProGluAlaIleHis

790 810 830

781 TTCAGTGGTGTGAAGATCTTTAGTAACTGCAGCTTCGAAGACTTTGCACATTTTATTTCA 840

PheSerGlyValLysIlePheSerAsnCysSerPheGluAspPheAlaHisPheIleSer

850 870 890

841 AAGCAGAAGTCCCAGTGTCTTCACAATCAGCCTCGCTTAGATCCTTTTTTCAAACAGCAA 900

LysGlnLysSerGlnCysLeuHisAsnGlnProArgLeuAspProPhePheLysGlnGln

910 930 950

901 GCAGTGTGTGGTAATGCAAAGCTGGAAGCAGGAGAGGAGTGTGACTGTGGGACTGAACAG 960

AlaValCysGlyAsnAlaLysLeuGluAlaGlyGluGluCysAspCysGlyThrGluGln

970 990 1010

961 GATTGTGCCCTTATTGGAGAAACATGCTGTGATATTGCCACATGTAGATTTAAAGCCGGT 1020

AspCysAlaLeuIleGlyGluThrCysCysAspIleAlaThrCysArgPheLysAlaGly

1030 1050 1070

1021 TCAAACTGTGCTGAAGGACCATGCTGCGAAAACTGTCTATTTATGTCAAAAGAAAGAATG 1080

SerAsnCysAlaGluGlyProCysCysGluAsnCysLeuPheMetSerLysGluArgMet

FIG.1C

```
          1090                1110                1130
            .         .         .         .         .         .

1081  TGTAGGCCTTCCTTTGAAGAATGCGACCTCCCTGAATATTGCAATGGATCATCTGCATCA 1140

      CysArgProSerPheGluGluCysAspLeuProGluTyrCysAsnGlySerSerAlaSer

          1150                1170                1190
            .         .         .         .         .         .

1141  TGCCCAGAAAACCACTATGTTCAGACTGGGCATCCGTGTGGACTGAATCAATGGATCTGT 1200

      CysProGluAsnHisTyrValGlnThrGlyHisProCysGlyLeuAsnGlnTrpIleCys

          1210                1230                1250
            .         .         .         .         .         .

1201  ATAGATGGAGTTTGTATGAGTGGGGATAAACAATGTACAGACACATTTGGCAAAGAAGTA 1260

      IleAspGlyValCysMetSerGlyAspLysGlnCysThrAspThrPheGlyLysGluVal

          1270                1290                1310
            .         .         .         .         .         .

1261  GAGTTTGGCCCTTCAGAATGTTATTCTCACCTTAATTCAAAGACTGATGTATCTGGAAAC 1320

      GluPheGlyProSerGluCysTyrSerHisLeuAsnSerLysThrAspValSerGlyAsn

          1330                1350                1370
            .         .         .         .         .         .

1321  TGTGGTATAAGTGATTCAGGATACACACAGTGTGAAGCTGACAATCTGCAGTGCGGAAAA 1380

      CysGlyIleSerAspSerGlyTyrThrGlnCysGluAlaAspAsnLeuGlnCysGlyLys

          1390                1410                1430
            .         .         .         .         .         .

1381  TTAATATGTAAATATGTAGGTAAATTTTTATTACAAATTCCAAGAGCCACTATTATTTAT 1440

      LeuIleCysLysTyrValGlyLysPheLeuLeuGlnIleProArgAlaThrIleIleTyr
```

FIG.1D

```
                1450                1470                1490
                 .    .    .    .    .    .    .    .    .    .

1441  GCCAACATAAGTGGACATCTCTGCATTGCTGTGGAATTTGCCAGTGATCATGCAGACAGC 1500

      AlaAsnIleSerGlyHisLeuCysIleAlaValGluPheAlaSerAspHisAlaAspSer

                1510                1530                1550
                 .    .    .    .    .    .    .    .    .    .

1501  CAAAAGATGTGGATAAAAGATGGAACTTCTTGTGGTTCAAATAAGGTTTGCAGGAATCAA 1560

      GlnLysMetTrpIleLysAspGlyThrSerCysGlySerAsnLysValCysArgAsnGln

                1570                1590                1610
                 .    .    .    .    .    .    .    .    .    .

1561  AGATGTGTGAGTTCTTCATACTTGGGTTATGATTGTACTACTGACAAATGCAATGATAGA 1620

      ArgCysValSerSerSerTyrLeuGlyTyrAspCysThrThrAspLysCysAsnAspArg

                1630                1650                1670
                 .    .    .    .    .    .    .    .    .    .

1621  GGTGTATGCAATAACAAAAAGCACTGTCACTGTAGTGCTTCATATTTACCTCCAGATTGC 1680

      GlyValCysAsnAsnLysLysHisCysHisCysSerAlaSerTyrLeuProProAspCys

                1690                1710                1730
                 .    .    .    .    .    .    .    .    .    .

1681  TCAGTTCAATCAGATCTATGGCCTGGTGGGAGTATTGACAGTGGCAATTTTCCACCTGTA 1740

      SerValGlnSerAspLeuTrpProGlyGlySerIleAspSerGlyAsnPheProProVal

                1750                1770                1790
                 .    .    .    .    .    .    .    .    .    .

1741  GCTATACCAGCCAGACTCCCTGAAAGGCGCTACATTGAGAACATTTACCATTCCAAACCA 1800

      AlaIleProAlaArgLeuProGluArgArgTyrIleGluAsnIleTyrHisSerLysPro
```

FIG.1E

```
          1810                1830                1850
           .   .   .   .   .   .   .   .   .   .   .   .
1801 ATGAGATGGCCATTTTTCTTATTCATTCCTTTCTTTATTATTTTCTGTGTACTGATTGCT 1860
     MetArgTrpProPhePheLeuPheIleProPhePheIleIlePheCysValLeuIleAla

          1870                1890                1910
           .   .   .   .   .   .   .   .   .   .   .   .
1861 ATAATGGTGAAAGTTAATTTCCAAAGGAAAAAATGGAGAACTGAGGACTATTCAAGCGAT 1920
     IleMetValLysValAsnPheGlnArgLysLysTrpArgThrGluAspTyrSerSerAsp

          1930                1950                1970
           .   .   .   .   .   .   .   .   .   .   .   .
1921 GAGCAACCTGAAAGTGAGAGTGAACCTAAAGGGTAGTCTGGACAACAGAGATGCCATGAT 1980
     GluGlnProGluSerGluSerGluProLysGly

          1990                2010                2030
           .   .   .   .   .   .   .   .   .   .   .   .
1981 ATCACTTCTTCTAGAGTAATTATCTGTGATGGATGGACACAAAAAAATGGAAAGAAAAGA 2040

          2050                2070                2090
           .   .   .   .   .   .   .   .   .   .   .   .
2041 ATGTACATTACCTGGTTTCCTGGGATTCAAACCTGCATATTGTGATTTTAATTTGACCAG 2100

          2110                2130                2150
           .   .   .   .   .   .   .   .   .   .   .   .
2101 AAAATATGATATATATGTATAATTTCACAGATAATTTACTTATTTAAAAATGCATGATAA 2160
```

FIG.1F

```
          2170                2190                2210
           .                   .                   .
2161 TGAGTTTTACATTACAAATTTCTGTTTTTTTAAAGTTATCTTACGCTATTTCTGTTGGTT 2220

          2230                2250                2270
           .                   .                   .
2221 AGTAGACACTAATTCTGTCAGTAGGGGCATGGTATAAGGAAATATCATAATGTAATGAGG 2280

          2290                2310                2330
           .                   .                   .
2281 TGGTACTATGATTAAAAGCCACTGTTACATTTCAAAAAAAAAAAAAAAAA 2330
```

FIG.1G

```
                10                  30                  50
                .         .         .         .         .         .

1   GGCACGAGCGATTATGTTGGCGCTACCTATCAAGGGAAGATGTGTGACAAGAACTATGCA 60

    GlyThrSerAspTyrValGlyAlaThrTyrGlnGlyLysMetCysAspLysAsnTyrAla

                70                  90                  110
                .         .         .         .         .         .

61  GGAGGAGTTGCTTTGCACCCCAAAGCCGTAACTCTGGAATCACTTGCAATTATTTTAGTT 120

    GlyGlyValAlaLeuHisProLysAlaValThrLeuGluSerLeuAlaIleIleLeuVal

                130                 150                 170
                .         .         .         .         .         .

121 CAGCTGCTGAGCCTCAGCATGGGGCTAGCGTATGACGACGTGAACAAGTGCCAGTGTGGC 180

    GlnLeuLeuSerLeuSerMetGlyLeuAlaTyrAspAspValAsnLysCysGlnCysGly

                190                 210                 230
                .         .         .         .         .         .

181 GTACCTGTCTGCGTGATGAACCCGGAAGCGCCTCACTCCAGCGGTGTCCGGGCCTTCAGT 240

    ValProValCysValMetAsnProGluAlaProHisSerSerGlyValArgAlaPheSer

                250                 270                 290
                .         .         .         .         .         .

241 AACTGCAGCATGGAGGACTTTTCCAAGTTTATCACAAGTCAAAGCTCCCACTGTCTGCAG 300

    AsnCysSerMetGluAspPheSerLysPheIleThrSerGlnSerSerHisCysLeuGln

                310                 330                 350
                .         .         .         .         .         .

301 AACCAGCCAACGCTACAGCCATCTTACAAGATGGCGGTCTGTGGGAATGGAGAGGTGGAA 360

    AsnGlnProThrLeuGlnProSerTyrLysMetAlaValCysGlyAsnGlyGluValGlu
```

FIG.2A

```
                370                 390                 410
                .   .   .   .   .   .   .   .   .   .   .   .

361 GAAGATGAAATTTGCGACTGTGGAAAGAAGGGCTGTGCAGAAATGCCCCCGCCATGCTGT 420

    GluAspGluIleCysAspCysGlyLysLysGlyCysAlaGluMetProProProCysCys

                430                 450                 470
                .   .   .   .   .   .   .   .   .   .   .   .

421 AACCCCGACACCTGTAAGCTGTCAGATGGCTCCGAGTGCTCCAGCGGGATATGCTGCAAC 480

    AsnProAspThrCysLysLeuSerAspGlySerGluCysSerSerGlyIleCysCysAsn

                490                 510                 530
                .   .   .   .   .   .   .   .   .   .   .   .

481 TCGTGCAAGCTGAAGCGGAAAGGGGAGGTTTGCAGGCTTGCCCAAGATGAGTGTGATGTC 540

    SerCysLysLeuLysArgLysGlyGluValCysArgLeuAlaGlnAspGluCysAspVal

                550                 570                 590
                .   .   .   .   .   .   .   .   .   .   .   .

541 ACAGAGTACTGCAACGGCACATCCGAAGTGTGTGAAGACTTCTTTGTTCAAAACGGTCAC 600

    ThrGluTyrCysAsnGlyThrSerGluValCysGluAspPhePheValGlnAsnGlyHis

                610                 630                 650
                .   .   .   .   .   .   .   .   .   .   .   .

601 CCATGTGACAATCGCAAGTGGATCTGTATTAACGGCACCTGTCAGAGTGGAGAACAGCAG 660

    ProCysAspAsnArgLysTrpIleCysIleAsnGlyThrCysGlnSerGlyGluGlnGln

                670                 690                 710
                .   .   .   .   .   .   .   .   .   .   .   .

661 TGCCAGGATCTATTTGGCATCGATGCAGGCTTTGGTTCAAGTGAATGTTTCTGGGAGCTG 720

    CysGlnAspLeuPheGlyIleAspAlaGlyPheGlySerSerGluCysPheTrpGluLeu
```

FIG.2B

730 750 770

721 AATTCCAAGAGCGACATATCTGGGAGCTGTGGAATCTCTGCTGGGGGATACAAGGAATGC 780

AsnSerLysSerAspIleSerGlySerCysGlyIleSerAlaGlyGlyTyrLysGluCys

790 810 830

781 CCACCTAATGACCGGATGTGTGGGAAAATAATATGTAAATACCAAAGTGAAAATATACTA 840

ProProAsnAspArgMetCysGlyLysIleIleCysLysTyrGlnSerGluAsnIleLeu

850 870 890

841 AAATTGAGGTCTGCCACTGTTATTTATGCCAATATAAGCGGGCATGTCTGCGTTTCCCTG 900

LysLeuArgSerAlaThrValIleTyrAlaAsnIleSerGlyHisValCysValSerLeu

910 930 950

901 GAATATCCCCAAGGTCATAATGAGAGCCAGAAGATGTGGGTGAGAGATGGAACCGTCTGC 960

GluTyrProGlnGlyHisAsnGluSerGlnLysMetTrpValArgAspGlyThrValCys

970 990 1010

961 GGGTCAAATAAGGTTTGCCAGAATCAAAAAATGTGTAGCAGACACTTTCTTGGGCTATGAT 1020

GlySerAsnLysValCysGlnAsnGlnLysCysValAlaAspThrPheLeuGlyTyrAsp

1030 1050 1070

1021 TGCAACCTGGAAAAATGCAACCACCATGGTGTATGTAATAACAAGAAGAACTGCCACTGT 1080

CysAsnLeuGluLysCysAsnHisHisGlyValCysAsnAsnLysLysAsnCysHisCys

FIG.2C

```
          1090                1110                1130
1081 GACCCCACATACTTACCTCCAGATTGTAAAAGAATGAAAGATTCATATCCTGGCGGGAGC 1140
     AspProThrTyrLeuProProAspCysLysArgMetLysAspSerTyrProGlyGlySer

          1150                1170                1190
1141 ATTGATAGTGGCAACAAGGAAAGGGCTGAACCCATCCCTGTACGGCCCTACATTGCAAGT 1200
     IleAspSerGlyAsnLysGluArgAlaGluProIleProValArgProTyrIleAlaSer

          1210                1230                1250
1201 CGTTACCGCTCCAAGTCTCCACGGTGGCCATTTTTCTTGATCATCCCTTTCTACGTTGTG 1260
     ArgTyrArgSerLysSerProArgTrpProPhePheLeuIleIleProPheTyrValVal

          1270                1290                1310
1261 ATCCTTGTCCTGATTGGGATGCTGGTAAAAGTCTATTCCCAAAGGATGAAATGGAGAATG 1320
     IleLeuValLeuIleGlyMetLeuValLysValTyrSerGlnArgMetLysTrpArgMet

          1330                1350                1370
1321 GATGACTTCTCAAGCGAAGAGCAATTTGAAAGTGAAAGTGAATCCAAAGACTAGTCTGGA 1380
     AspAspPheSerSerGluGluGlnPheGluSerGluSerGluSerLysAsp

          1390                1410                1430
1381 CAGATTCCACAATGTCACAAGTAATTCTCTTCAGTGGACAGAAAAAAAAGTGGAAAAGAA 1440

          1450                1470                1490
1441 AAGCCTATGCATTATCTTGCCTGAAAGTCAAGCCTGCATATCGTGGTCTCCATCAGGCCA 1500

          1510                1530                1550
1501 GAAATCATATCTCTCCATTACACATGTATGATACATATGTGTGTATATTATTCCATAAAT 1560
```

FIG.2D

```
          1570                1590                1610
1561 GATTTACTTGTAAGAAATGAATGATTATGAATTTCATATTATACTTTGATATTTTACCCT 1620

          1630                1650                1670
1621 ATTTCTGGTAGTCGGTAGTCATCAATTGTATTTTCTAGTAGGTACATTATAGAAAAGGCT 1680

          1690
1681 ATAAGAAAATAAATGTGGTACCA 1703
```

FIG.2E

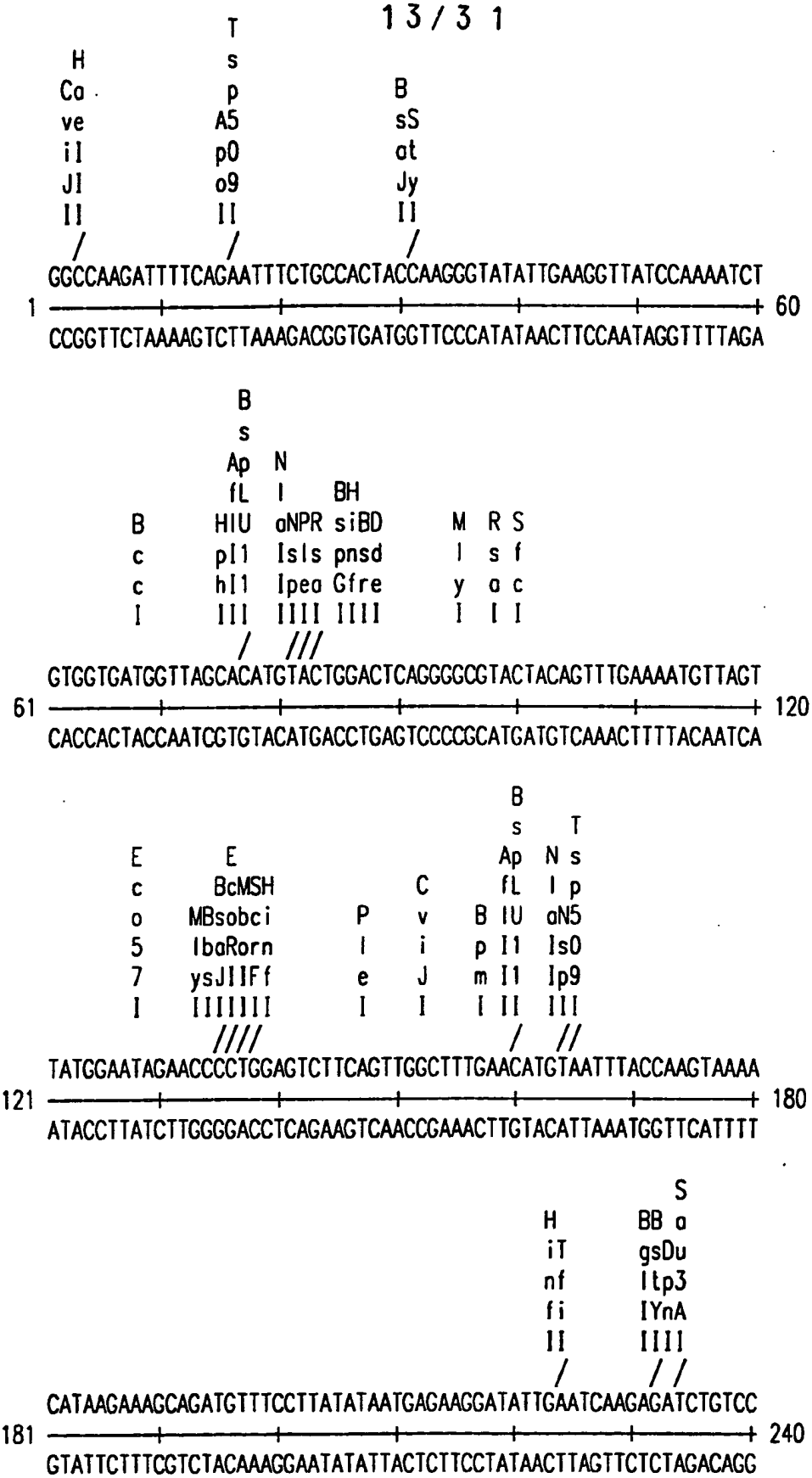

GGCCAAGATTTTCAGAATTTCTGCCACTACCAAGGGTATATTGAAGGTTATCCAAAATCT
1
60
CCGGTTCTAAAAGTCTTAAAGACGGTGATGGTTCCCATATAACTTCCAATAGGTTTTAGA
GTGGTGATGGTTAGCACATGTACTGGACTCAGGGGCGTACTACAGTTTGAAAATGTTAGT
61
120
CACCACTACCAATCGTGTACATGACCTGAGTCCCCGCATGATGTCAAACTTTTACAATCA
TATGGAATAGAACCCCTGGAGTCTTCAGTTGGCTTTGAACATGTAATTTACCAAGTAAAA
121
180
ATACCTTATCTTGGGGACCTCAGAAGTCAACCGAAACTTGTACATTAAATGGTTCATTTT
CATAAGAAAGCAGATGTTTCCTTATATAATGAGAAGGATATTGAATCAAGAGATCTGTCC
181
240
GTATTCTTTCGTCTACAAAGGAATATATTACTCTTCCTATAACTTAGTTCTCTAGACAGG

FIG.3A

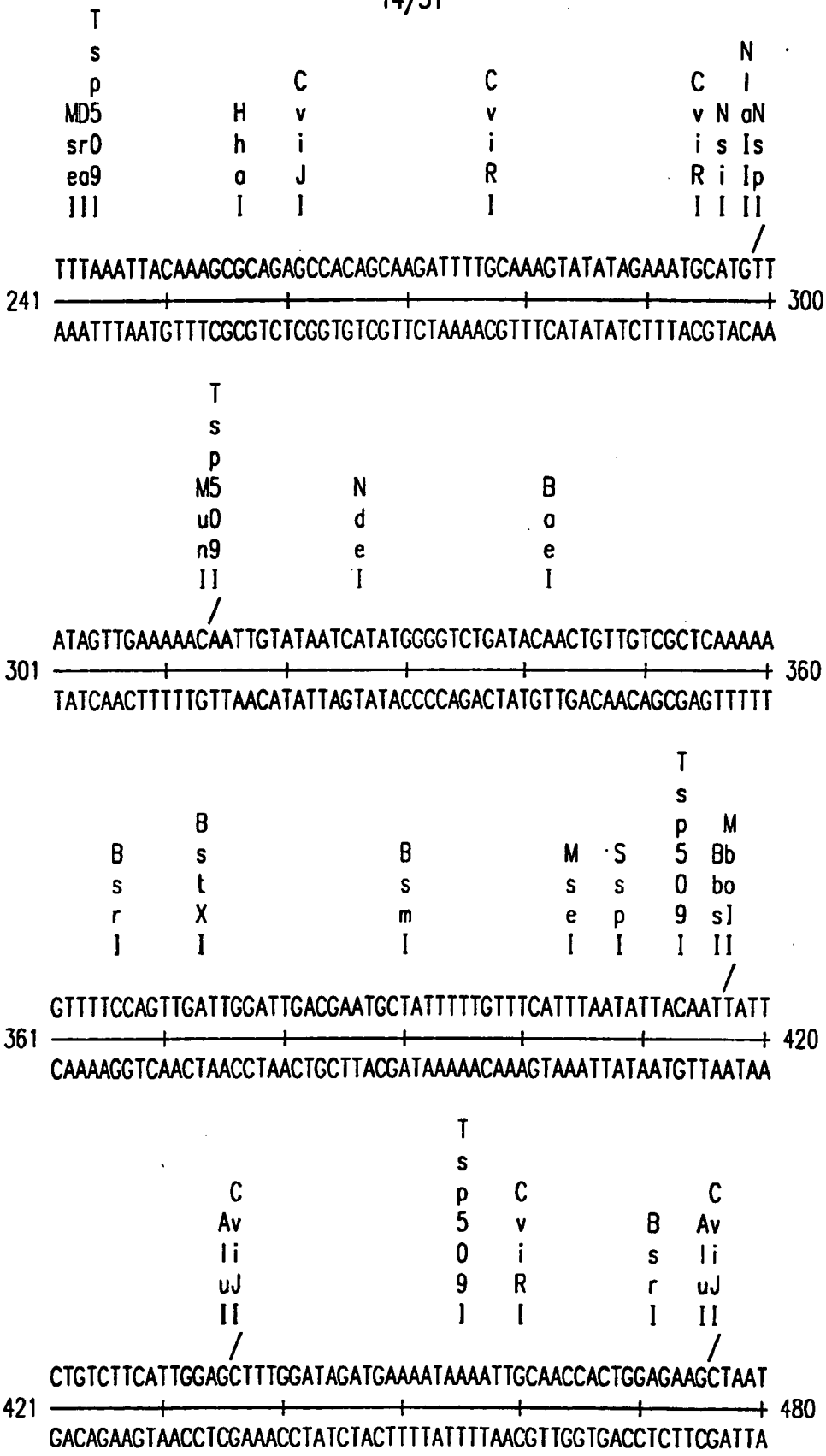
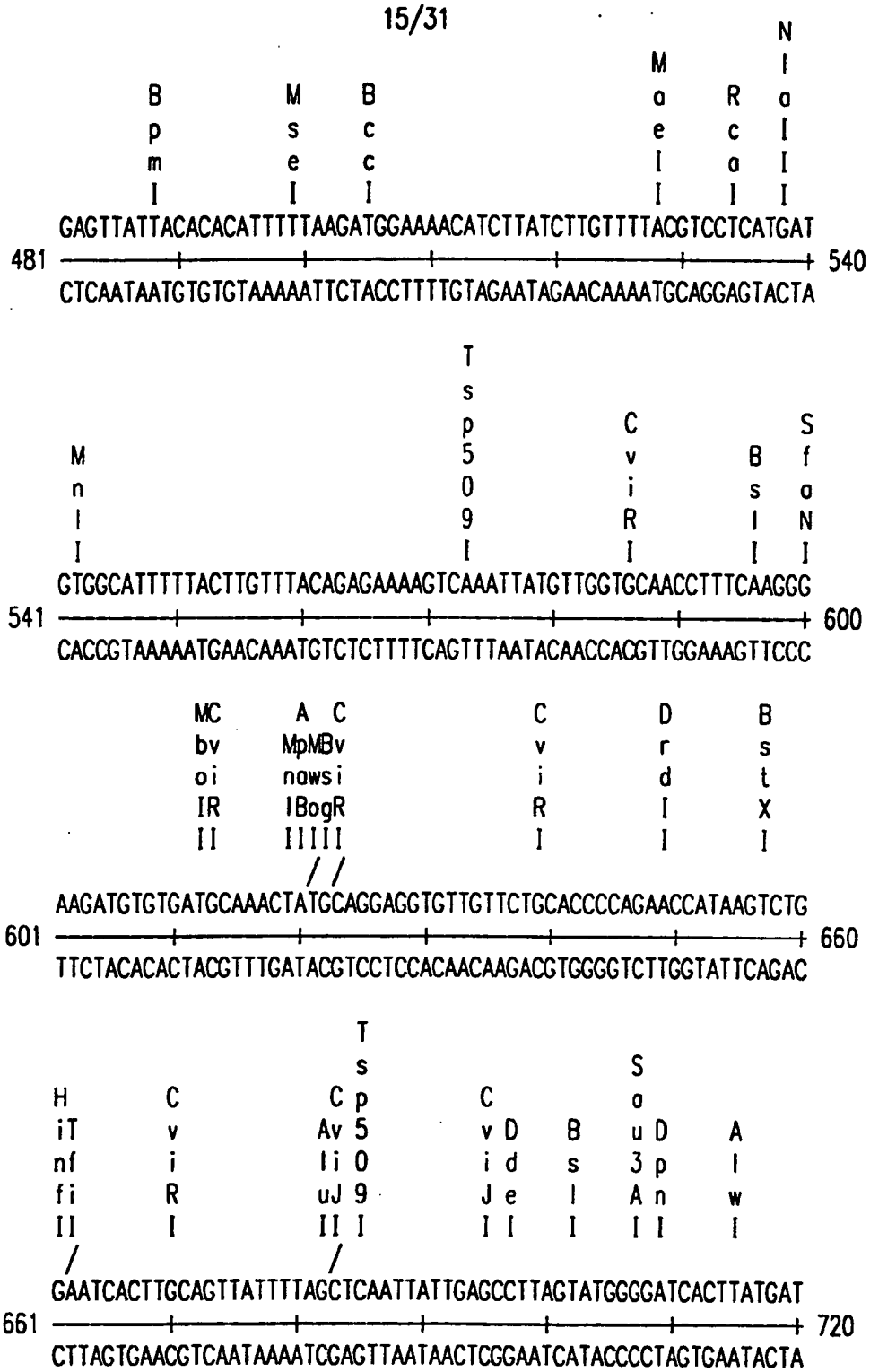

TspMD5sr0ea9III
HhaI
CviJI
CviRI
CviNsRiII
NIaNIsIpII
TTTAAATTACAAAGCGCAGAGCCACAGCAAGATTTTGCAAAGTATATAGAAATGCATGTT
241
300
AAATTTAATGTTTCGCGTCTCGGTGTCGTTCTAAAACGTTTCATATATCTTTACGTACAA
TspM5u0n9II
NdeI
BaeI
ATAGTTGAAAAACAATTGTATAATCATATGGGGTCTGATACAACTGTTGTCGCTCAAAAA
301
360
TATCAACTTTTTGTTAACATATTAGTATACCCCAGACTATGTTGACAACAGCGAGTTTTT
BsrI
BstXI
BsmI
MseI
SspI
TspM5091II
BbsI
GTTTTCCAGTTGATTGGATTGACGAATGCTATTTTTGTTTCATTTAATATTACAATTATT
361
420
CAAAAGGTCAACTAACCTAACTGCTTACGATAAAAACAAAGTAAATTATAATGTTAATAA
CviIIuJII
TspM5091I
CviRI
BsrI
CviIIuJII
CTGTCTTCATTGGAGCTTTGGATAGATGAAAATAAAATTGCAACCACTGGAGAAGCTAAT
421
480
GACAGAAGTAACCTCGAAACCTATCTACTTTTATTTTAACGTTGGTGACCTCTTCGATTA

FIG.3B


BpmI
MseI
BccI
MaeI
RcaI
NlaIII
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481
540
CTCAATAATGTGTGTAAAAATTCTACCTTTTGTAGAATAGAACAAAATGCAGGAGTACTA
MnlI
Tsp509I
CviRI
BslI
SfaNI
GTGGCATTTTTACTTGTTTACAGAGAAAAGTCAAATTATGTTGGTGCAACCTTTCAAGGG
541
600
CACCGTAAAAATGAACAAATGTCTCTTTTCAGTTTAATACAACCACGTTGGAAAGTTCCC
MCbvoiIR
AcMpMBvnawsiIBogRIIIII
CviRI
DrdI
BstXI
AAGATGTGTGATGCAAACTATGCAGGAGGTGTTGTTCTGCACCCCAGAACCATAAGTCTG
601
660
TTCTACACACTACGTTTGATACGTCCTCCACAACAAGACGTGGGGTCTTGGTATTCAGAC
HiTnfiII
CviRI
CAviIiuJII
Tsp509I
CvidJeII
BsmI
SouD3pAnII
AlwI
GAATCACTTGCAGTTATTTTAGCTCAATTATTGAGCCTTAGTATGGGGATCACTTATGAT
661
720
CTTAGTGAACGTCAATAAAATCGAGTTAATAACTCGGAATCATACCCCTAGTGAATACTA

FIG.3C

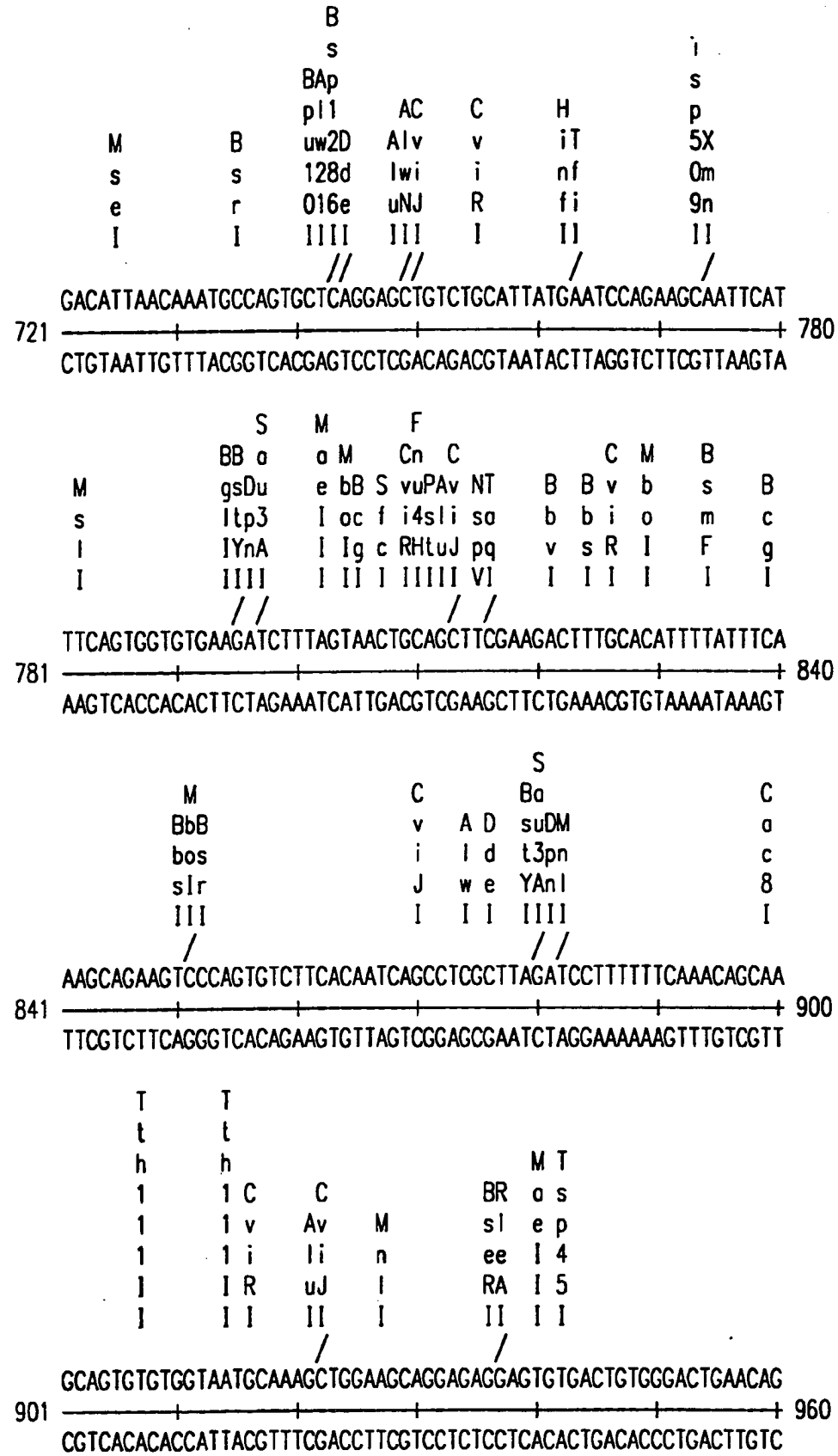

GACATTAACAAATGCCAGTGCTCAGGAGCTGTCTGCATTATGAATCCAGAAGCAATTCAT
721 780
CTGTAATTGTTTACGGTCACGAGTCCTCGACAGACGTAATACTTAGGTCTTCGTTAAGTA
TTCAGTGGTGTGAAGATCTTTAGTAACTGCAGCTTCGAAGACTTTGCACATTTTATTTCA
781 840
AAGTCACCACACTTCTAGAAATCATTGACGTCGAAGCTTCTGAAACGTGTAAAATAAAGT
AAGCAGAAGTCCCAGTGTCTTCACAATCAGCCTCGCTTAGATCCTTTTTTCAAACAGCAA
841 900
TTCGTCTTCAGGGTCACAGAAGTGTTAGTCGGAGCGAATCTAGGAAAAAAGTTTGTCGTT
GCAGTGTGTGGTAATGCAAAGCTGGAAGCAGGAGAGGAGTGTGACTGTGGGACTGAACAG
901 960
CGTCACACACCATTACGTTTCGACCTTCGTCCTCTCCTCACACTGACACCCTGACTTGTC

FIG.3D

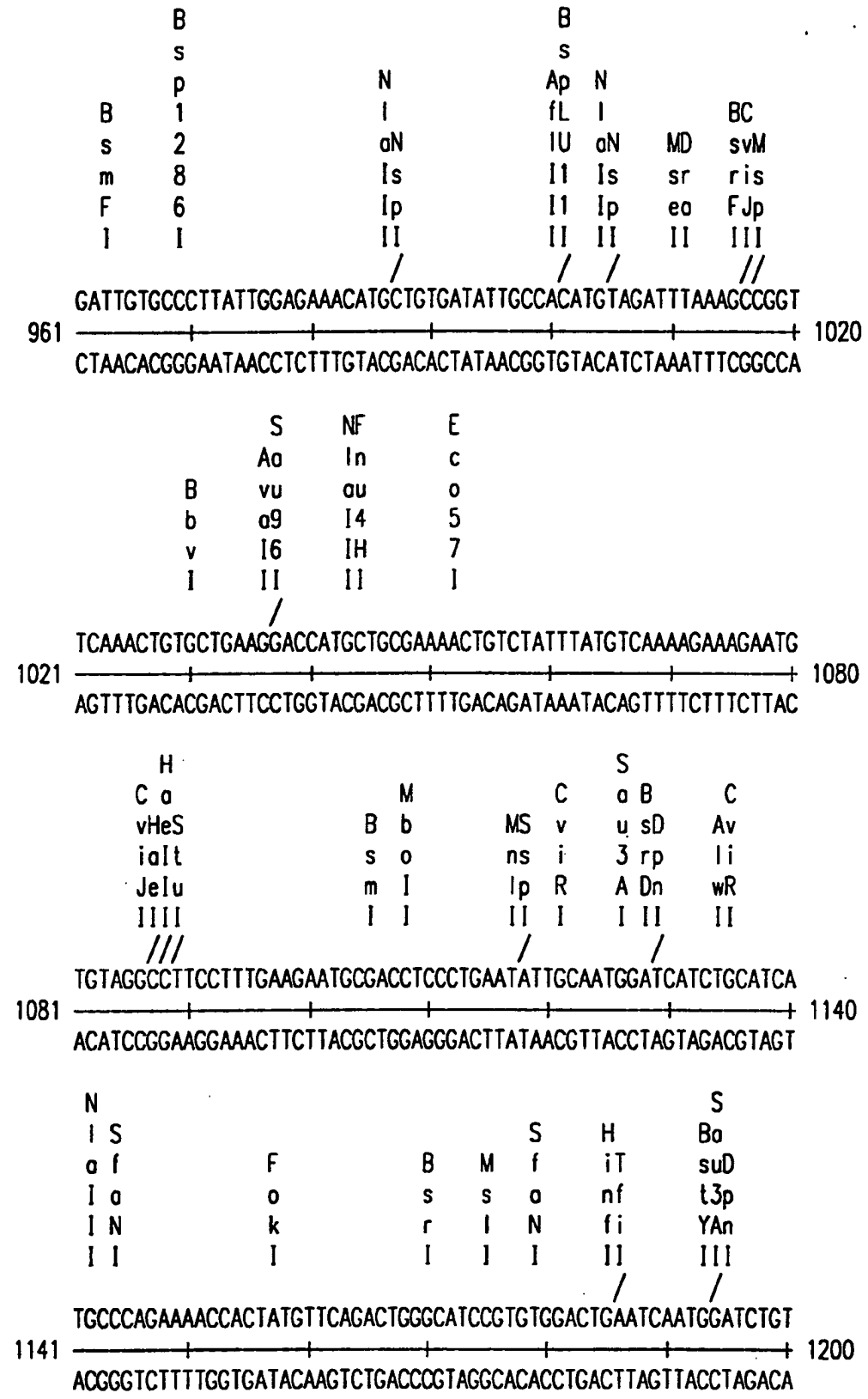

GATTGTGCCCTTATTGGAGAAACATGCTGTGATATTGCCACATGTAGATTTAAAGCCGGT
961
1020
CTAACACGGGAATAACCTCTTTGTACGACACTATAACGGTGTACATCTAAATTTCGGCCA
TCAAACTGTGCTGAAGGACCATGCTGCGAAAACTGTCTATTTATGTCAAAAGAAAGAATG
1021
1080
AGTTTGACACGACTTCCTGGTACGACGCTTTTGACAGATAAATACAGTTTTCTTTCTTAC
TGTAGGCCTTCCTTTGAAGAATGCGACCTCCCTGAATATTGCAATGGATCATCTGCATCA
1081
1140
ACATCCGGAAGGAAACTTCTTACGCTGGAGGGACTTATAACGTTACCTAGTAGACGTAGT
TGCCCAGAAAACCACTATGTTCAGACTGGGCATCCGTGTGGACTGAATCAATGGATCTGT
1141
1200
ACGGGTCTTTTGGTGATACAAGTCTGACCCGTAGGCACACCTGACTTAGTTACCTAGACA

FIG.3E

AlwI, BccI, Bsp1407I, RsaI, Eco57I

```
     ATAGATGGAGTTTGTATGAGTGGGGATAAACAATGTACAGACACATTTGGCAAAGAAGTA
1201 ------------------------------------------------------------ 1260
     TATCTACCTCAAACATACTCACCCCTATTTGTTACATGTCTGTGTAAACCGTTTCTTCAT
```

Sau96I, CviJI, HaeIII, HphI, MseI, Tsp509I, BaeI

```
     GAGTTTGGCCCTTCAGAATGTTATTCTCACCTTAATTCAAAGACTGATGTATCTGGAAAC
1261 ------------------------------------------------------------ 1320
     CTCAAACCGGGAAGTCTTACAATAAGAGTGGAATTAAGTTTCTGACTACATAGACCTTTG
```

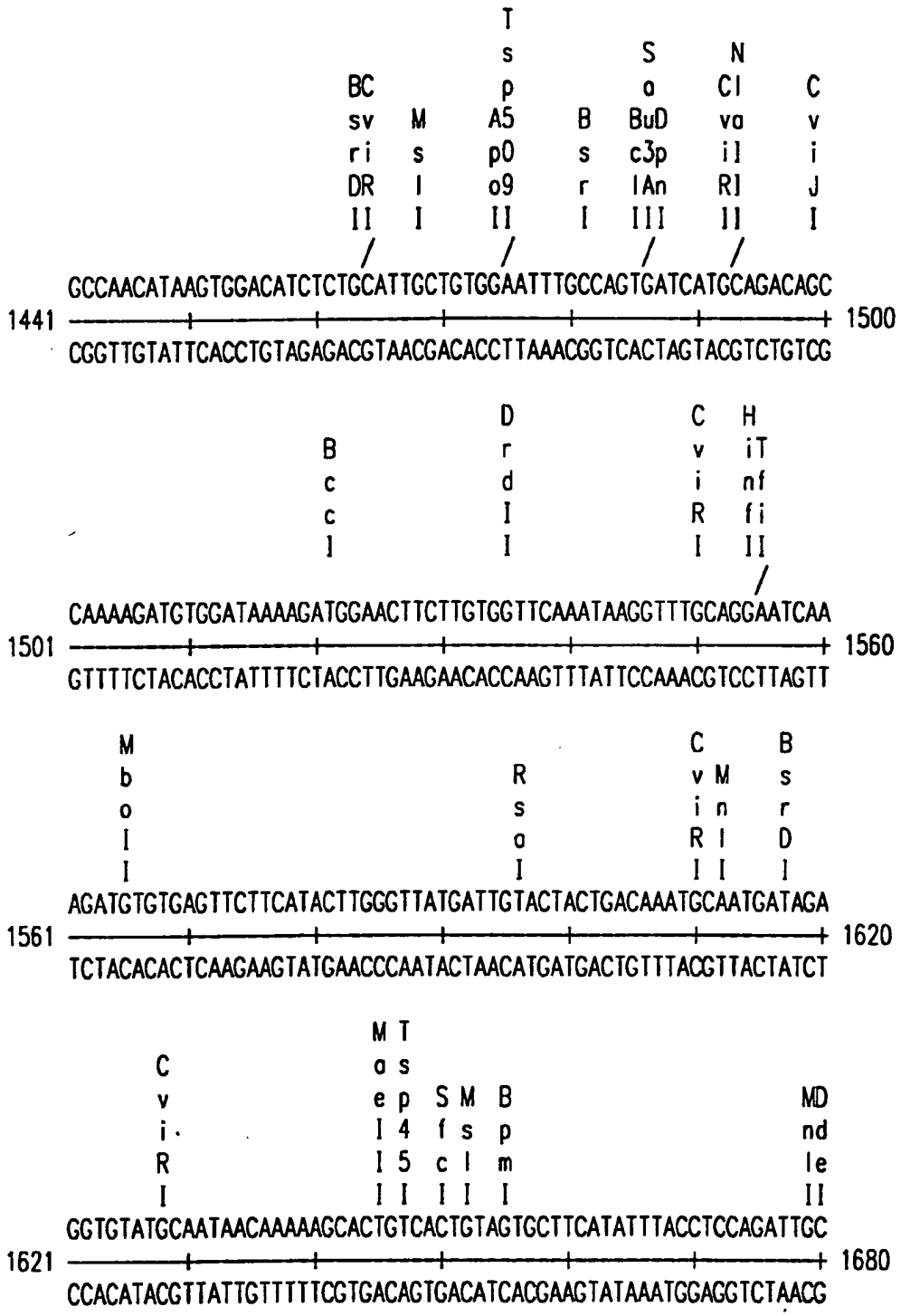
HinfI, TfiI, DraIII, DraII, AluI, CviJI, SfcI, CviRI, PstI, AciI, Tsp509I

```
     TGTGGTATAAGTGATTCAGGATACACACAGTGTGAAGCTGACAATCTGCAGTGCGGAAAA
1321 ------------------------------------------------------------ 1380
     ACACCATATTCACTAAGTCCTATGTGTGTCACACTTCGACTGTTAGACGTCACGCCTTTT
```

MseI, VspI, ApoI, Tsp509I, ApoI, Tsp509I, CviJI

```
     TTAATATGTAAATATGTAGGTAAATTTTTATTACAAATTCCAAGAGCCACTATTATTTAT
1381 ------------------------------------------------------------ 1440
     AATTATACATTTATACATCCATTTAAAAATAATGTTTAAGGTTCTCGGTGATAATAAATA
```

FIG.3F


T
s
p
A5
pO
o9
II
S
a
BuD
c3p
lAn
III
N
Cl
va
il
RI
II
C
v
i
J
I
BC
sv
ri
DR
II
M
s
I
I
B
s
r
I
I
GCCAACATAAGTGGACATCTCTGCATTGCTGTGGAATTTGCCAGTGATCATGCAGACAGC
1441
1500
CGGTTGTATTCACCTGTAGAGACGTAACGACACCTTAAACGGTCACTAGTACGTCTGTCG
D
r
d
I
I
C
v
i
R
I
H
iT
nf
fi
II
B
c
c
l
CAAAAGATGTGGATAAAAGATGGAACTTCTTGTGGTTCAAATAAGGTTTGCAGGAATCAA
1501
1560
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M
b
o
I
I
R
s
o
I
C
v M
i n
R I
I I
B
s
r
D
I
AGATGTGTGAGTTCTTCATACTTGGGTTATGATTGTACTACTGACAAATGCAATGATAGA
1561
1620
TCTACACACTCAAGAAGTATGAACCCAATACTAACATGATGACTGTTTACGTTACTATCT
M T
a s
e p S M B
I 4 f s p
I 5 c I m
I I I I I
C
v
i
R
I
MD
nd
le
II
GGTGTATGCAATAACAAAAAGCACTGTCACTGTAGTGCTTCATATTTACCTCCAGATTGC
1621
1680
CCACATACGTTATTGTTTTTCGTGACAGTGACATCACGAAGTATAAATGGAGGTCTAACG

FIG.3G

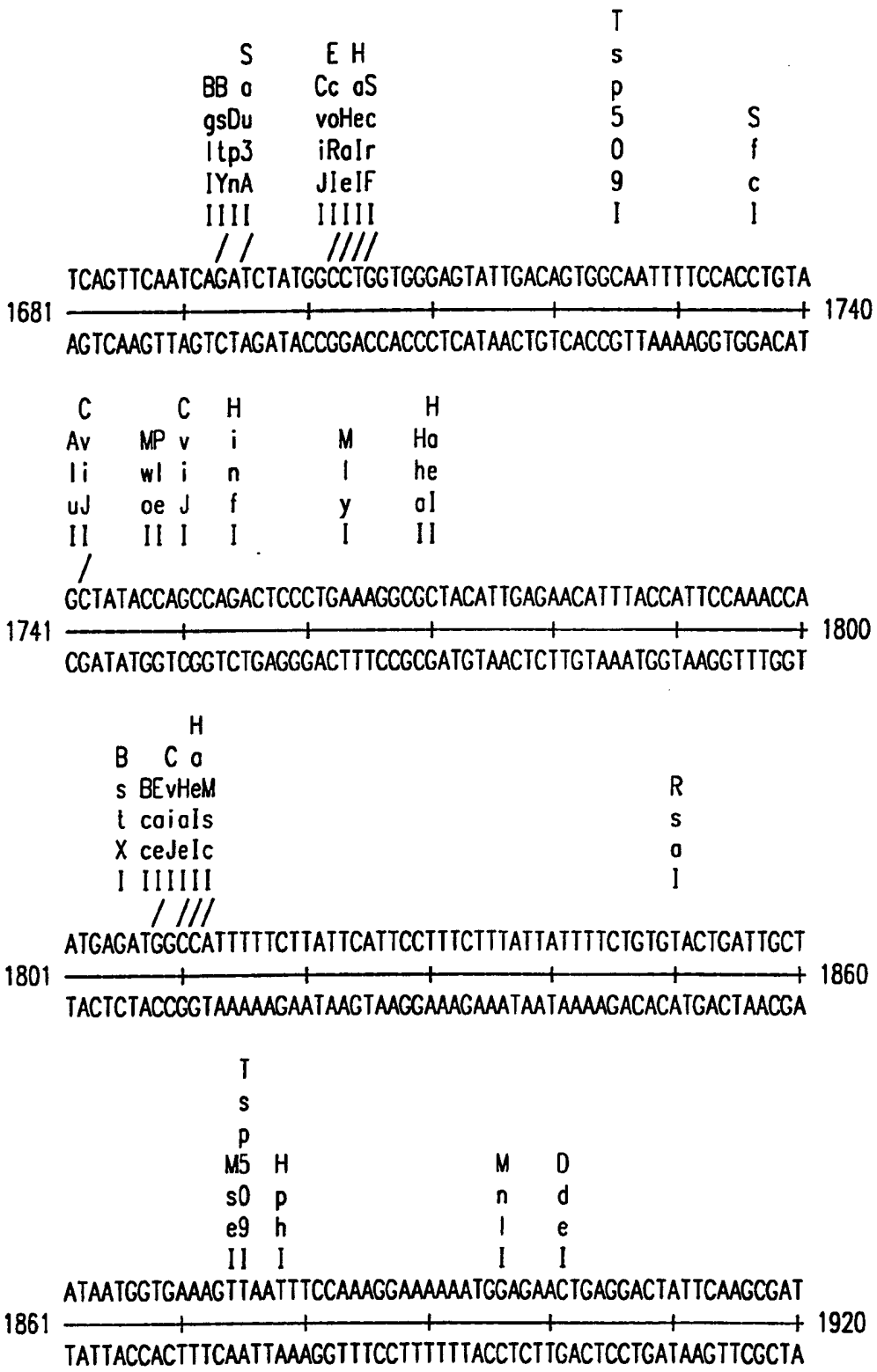
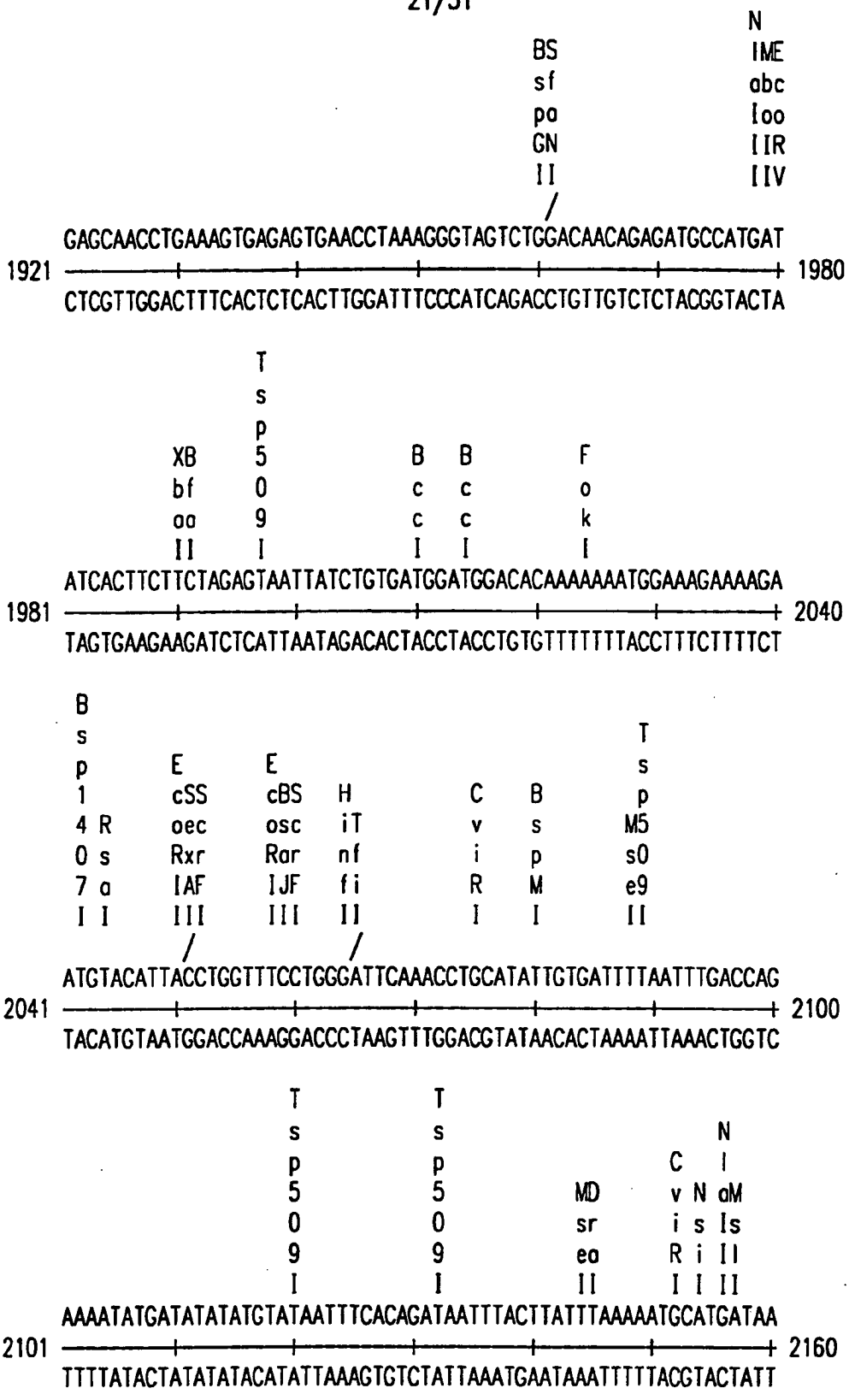

TCAGTTCAATCAGATCTATGGCCTGGTGGGAGTATTGACAGTGGCAATTTTCCACCTGTA
1681
1740
AGTCAAGTTAGTCTAGATACCGGACCACCCTCATAACTGTCACCGTTAAAAGGTGGACAT
GCTATACCAGCCAGACTCCCTGAAAGGCGCTACATTGAGAACATTTACCATTCCAAACCA
1741
1800
CGATATGGTCGGTCTGAGGGACTTTCCGCGATGTAACTCTTGTAAATGGTAAGGTTTGGT
ATGAGATGGCCATTTTTCTTATTCATTCCTTTCTTTATTATTTTCTGTGTACTGATTGCT
1801
1860
TACTCTACCGGTAAAAAGAATAAGTAAGGAAAGAAATAATAAAAGACACATGACTAACGA
ATAATGGTGAAAGTTAATTTCCAAAGGAAAAAATGGAGAACTGAGGACTATTCAAGCGAT
1861
1920
TATTACCACTTTCAATTAAAGGTTTCCTTTTTTACCTCTTGACTCCTGATAAGTTCGCTA

FIG.3H


N
IME
abc
loo
IIR
IIV
BS
sf
po
GN
II
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1921
1980
CTCGTTGGACTTTCACTCTCACTTGGATTTCCCATCAGACCTGTTGTCTCTACGGTACTA
T
s
p
XB
bf
oo
II
5
0
9
I
B
c
c
I
B
c
c
I
F
o
k
I
ATCACTTCTTCTAGAGTAATTATCTGTGATGGATGGACACAAAAAAATGGAAAGAAAAGA
1981
2040
TAGTGAAGAAGATCTCATTAATAGACACTACCTACCTGTGTTTTTTTACCTTTCTTTTCT
B
s
p
1
4 R
0 s
7 a
I I
E
cSS
oec
Rxr
IAF
III
E
cBS
osc
Rar
IJF
III
H
iT
nf
fi
II
C
v
i
R
I
B
s
p
M
I
T
s
p
M5
s0
e9
II
ATGTACATTACCTGGTTTCCTGGGATTCAAACCTGCATATTGTGATTTTAATTTGACCAG
2041
2100
TACATGTAATGGACCAAAGGACCCTAAGTTTGGACGTATAACACTAAAATTAAACTGGTC
T
s
p
5
0
9
I
T
s
p
5
0
9
I
MD
sr
ea
II
C
v
i
R
N
s
i
I
N
aM
Is
II
II
AAAATATGATATATATGTATAATTTCACAGATAATTTACTTATTTAAAAATGCATGATAA
2101
2160
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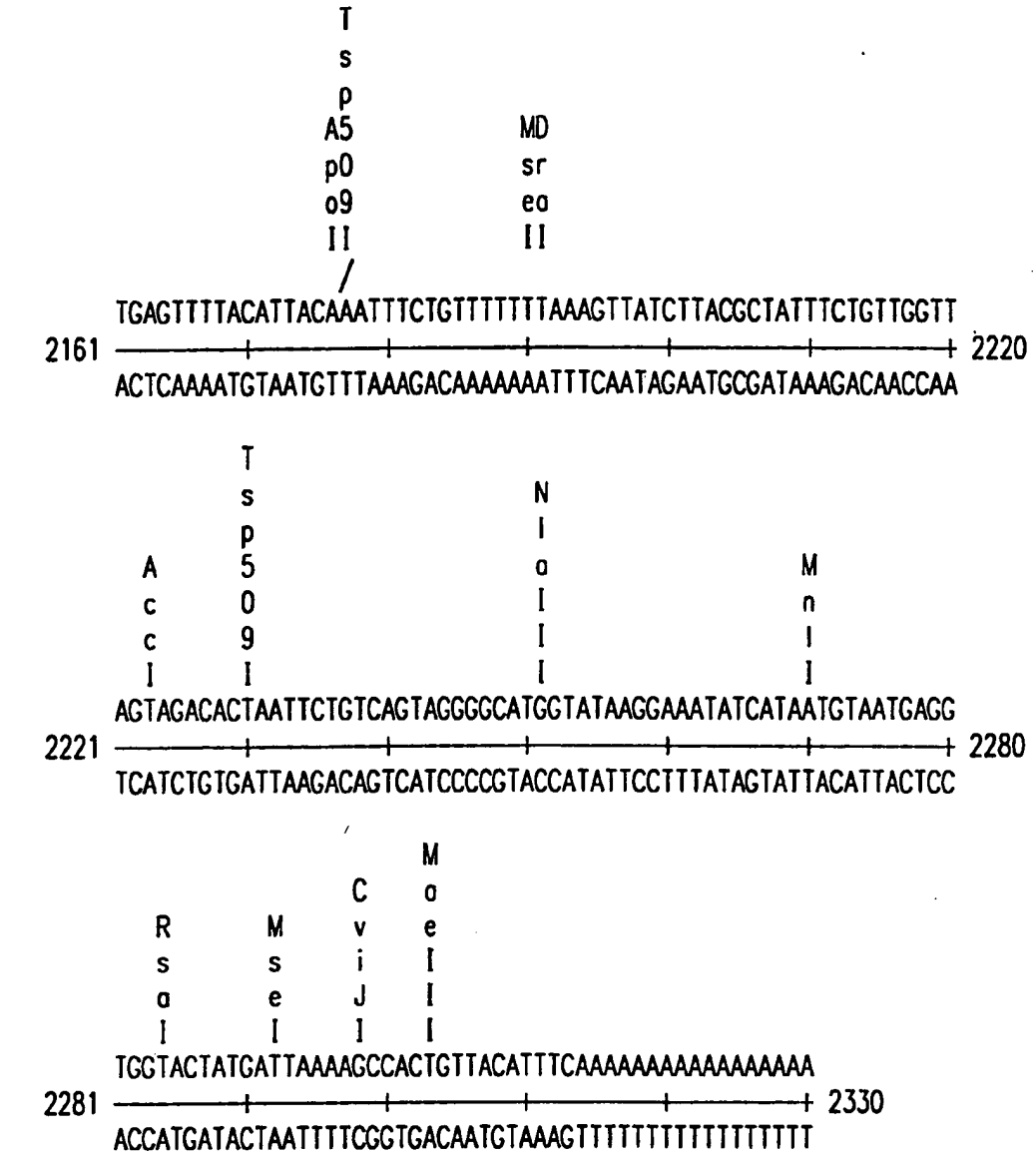
FIG.31



Enzymes that do cut:

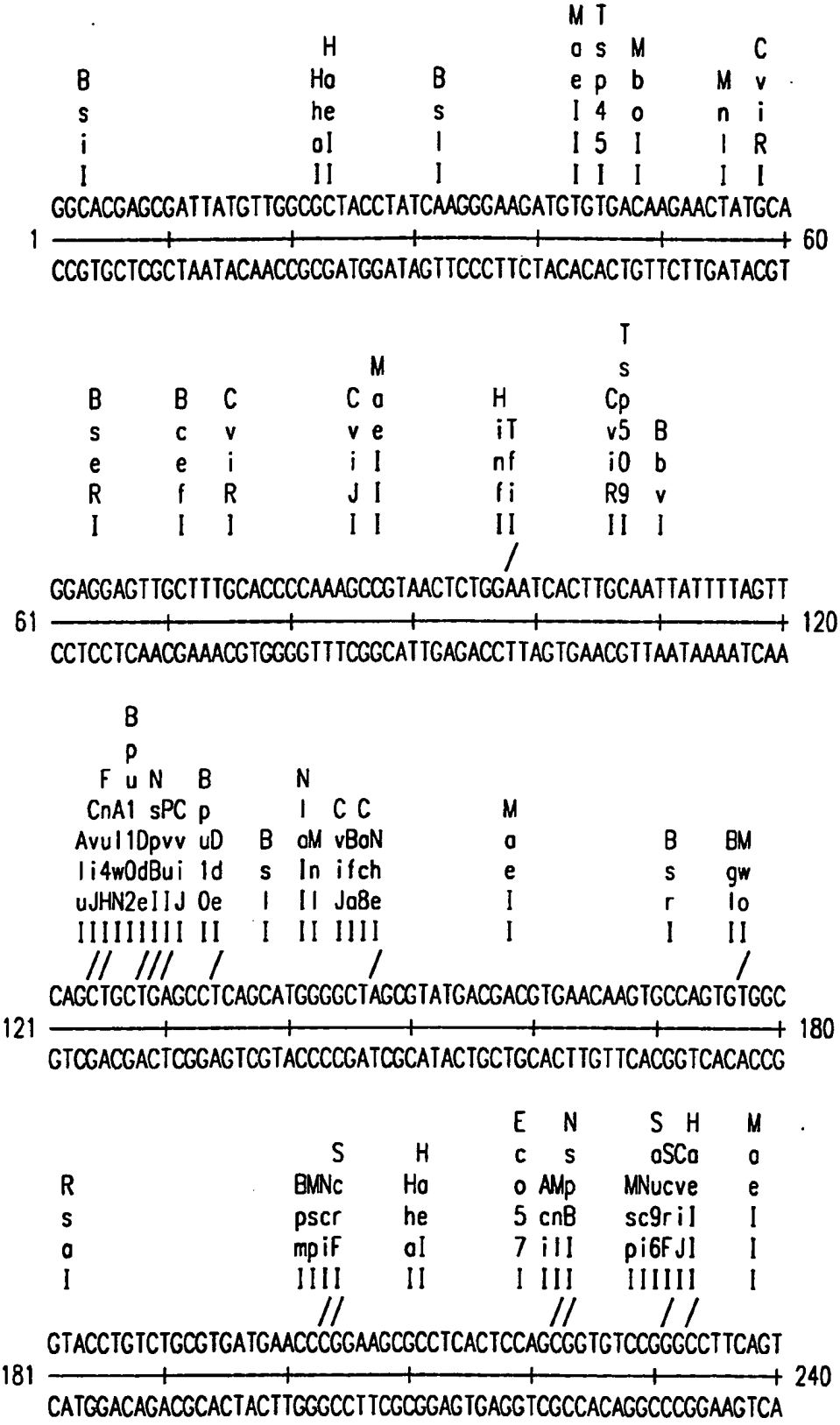
AccI	AciI	AflIII	AluI	AlwI	Alw21I	AlwNI	ApaBI
ApoI	AvaII	BaeI	BbsI	BbvI	BccI	BcgI	BclI
BfaI	BglII	BpmI	Bpu10I	BsaJI	BseRI	BsgI	BslI
BsmI	BsmFI	Bsp1286I	Bsp1407I	BspGI	BspLU11I	BspMI	BsrI
BsrDI	BsrFI	BstXI	BstYI	Cac8I	CviJI	CviRI	DdeI
DpnI	DraI	DraIII	DrdII	EaeI	Eco57I	EcoRII	EcoRV
Fnu4HI	FokI	HaeI	HaeII	HaeIII	HhaI	HinfI	HphI
MaeII	MaeIII	MboII	MlyI	MnlI	MscI	MseI	MslI
MspI	MunI	MwoI	NdeI	NlaIII	NsiI	NspI	NspV
PleI	PstI	RcaI	RleAI	RsaI	Sau96I	Sau3AI	ScrFI
SexAI	SfaNI	SfcI	SspI	StuI	StyI	TaqI	TfiI
Tsp45I	Tsp509I	Tth111I	VspI	XbaI	XmnI		

FIG.3J

Enzymes that do not cut:

AatII	AflII	AgeI	Alw44I	ApaI	AscI	AvaI	AvrII
BamHI	BanI	BanII	Bce83I	BcefI	BglI	Bpu1102I	BsaI
BsaAI	BsaBI	BsaHI	BsaWI	BscGI	BsiI	BsiEI	BsiWI
BsmAI	BspEI	BsrBI	BssHII	Bst1107I	BstEII	Bsu36I	ClaI
DrdI	DsaI	EagI	Eam1105I	EarI	EciI	Eco47III	Eco105I
EcoNI	Eco0109I	EcoRI	Esp3I	FauI	FseI	FspI	GdiII
HgaI	HgiEII	HincII	HindIII	HpaI	KpnI	MluI	MmeI
NaeI	NarI	NciI	NcoI	NheI	NlaIV	NotI	NruI
NspBII	PacI	Pfl1108I	PflMI	PmeI	PmlI	PshAI	Psp5II
Psp1406I	PvuI	PvuII	RsrII	SacI	SacII	SalI	SapI
ScaI	SfiI	SgrAI	SmaI	SpeI	SphI	SrfI	Sse8387I
SwaI	TaqII	TaqII	ThaI	Tth111I	XcmI	XhoI	

FIG.3K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1
60
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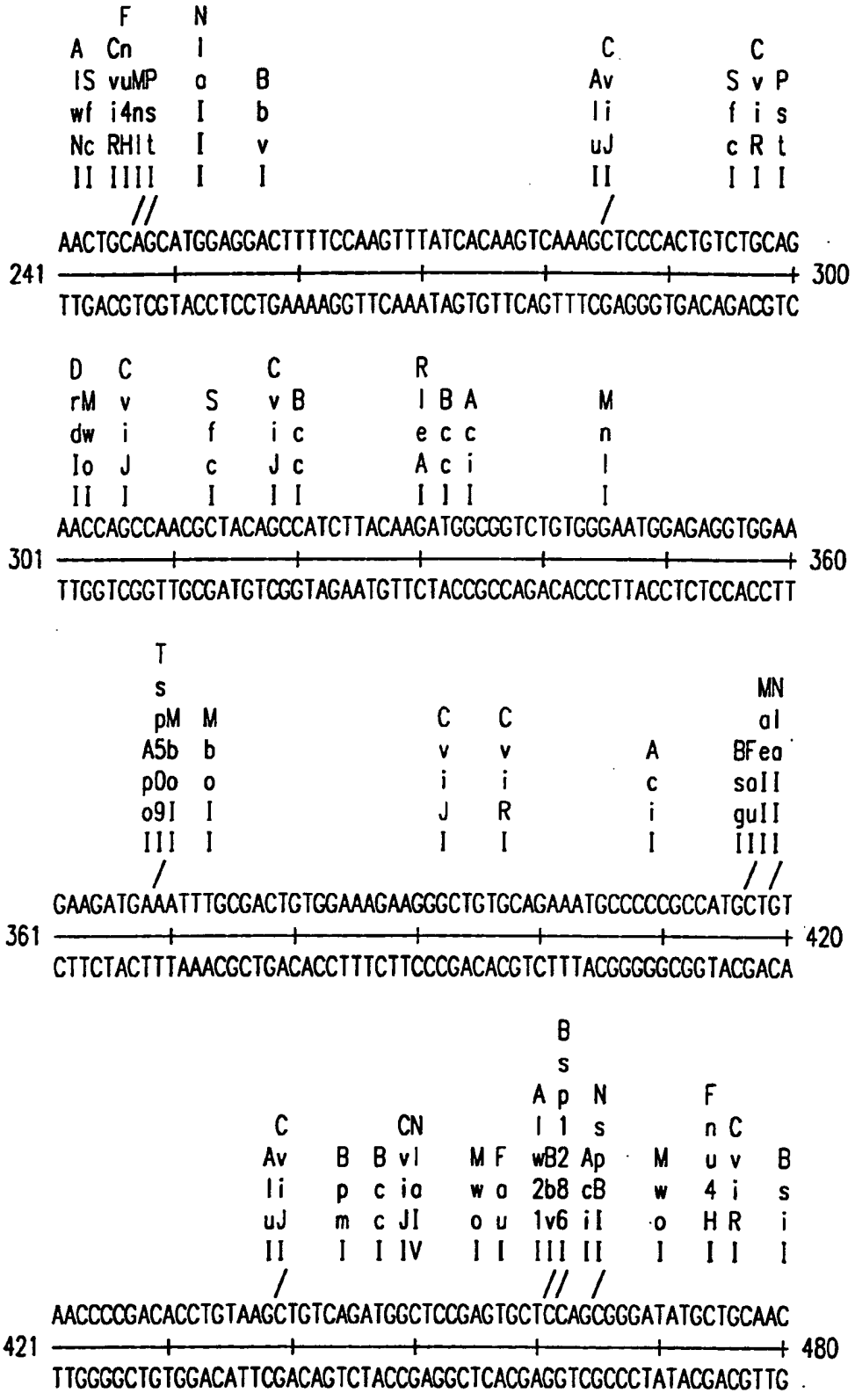
GGAGGAGTTGCTTTGCACCCCAAAGCCGTAACTCTGGAATCACTTGCAATTATTTTAGTT
61
120
CCTCCTCAACGAAACGTGGGGTTTCGGCATTGAGACCTTAGTGAACGTTAATAAAATCAA
CAGCTGCTGAGCCTCAGCATGGGGCTAGCGTATGACGACGTGAACAAGTGCCAGTGTGGC
121
180
GTCGACGACTCGGAGTCGTACCCCGATCGCATACTGCTGCACTTGTTCACGGTCACACCG
GTACCTGTCTGCGTGATGAACCCGGAAGCGCCTCACTCCAGCGGTGTCCGGGCCTTCAGT
181
240
CATGGACAGACGCACTACTTGGGCCTTCGCGGAGTGAGGTCGCCACAGGCCCGGAAGTCA

FIG.4A


AACTGCAGCATGGAGGACTTTTCCAAGTTTATCACAAGTCAAAGCTCCCACTGTCTGCAG
241
300
TTGACGTCGTACCTCCTGAAAAGGTTCAAATAGTGTTCAGTTTCGAGGGTGACAGACGTC
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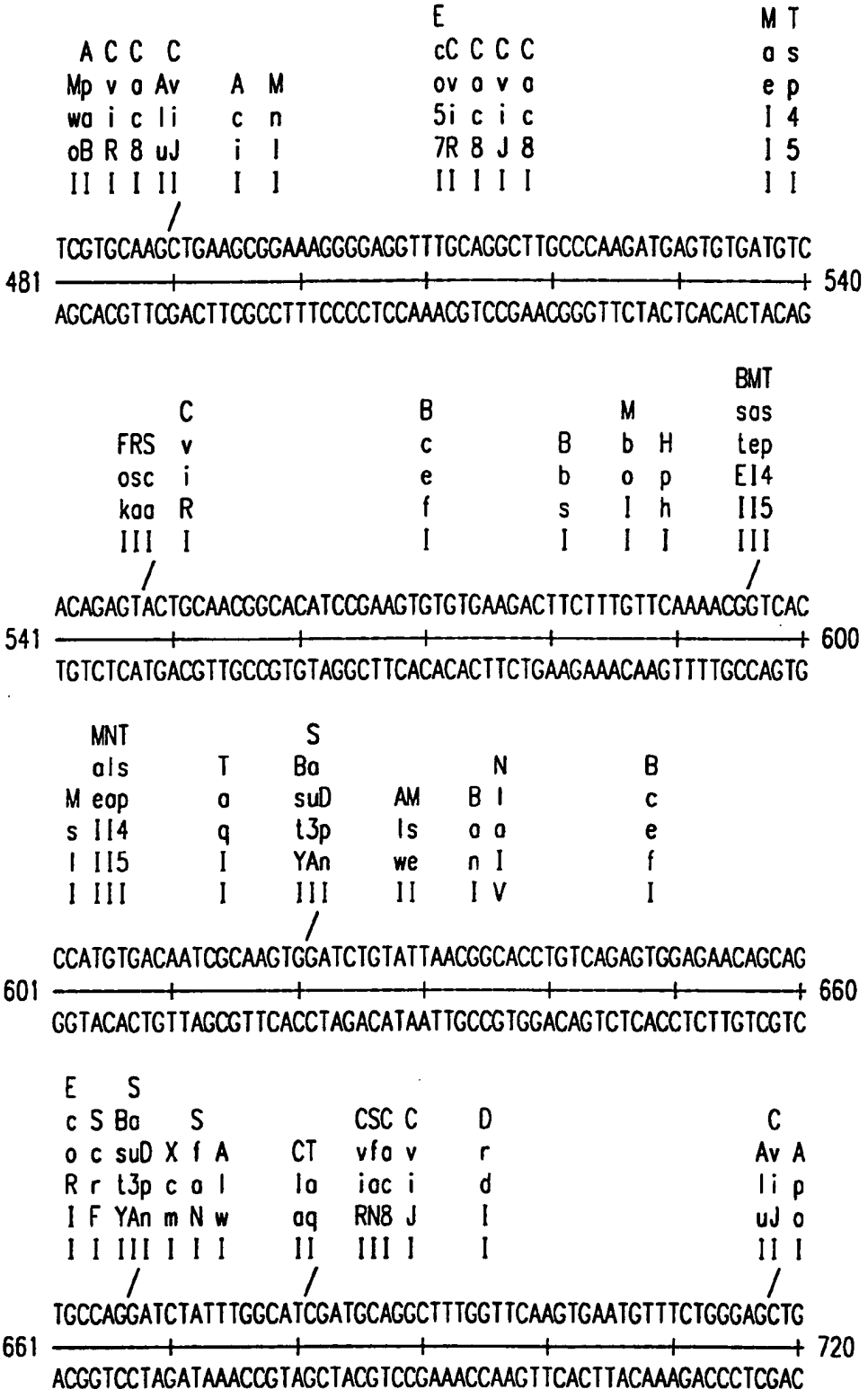
301
360
TTGGTCGGTTGCGATGTCGGTAGAATGTTCTACCGCCAGACACCCTTACCTCTCCACCTT
GAAGATGAAATTTGCGACTGTGGAAAGAAGGGCTGTGCAGAAATGCCCCCGCCATGCTGT
361
420
CTTCTACTTTAAACGCTGACACCTTTCTTCCCGACACGTCTTTACGGGGGCGGTACGACA
AACCCCGACACCTGTAAGCTGTCAGATGGCTCCGAGTGCTCCAGCGGGATATGCTGCAAC
421
480
TTGGGGCTGTGGACATTCGACAGTCTACCGAGGCTCACGAGGTCGCCCTATACGACGTTG

FIG.4B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481
540
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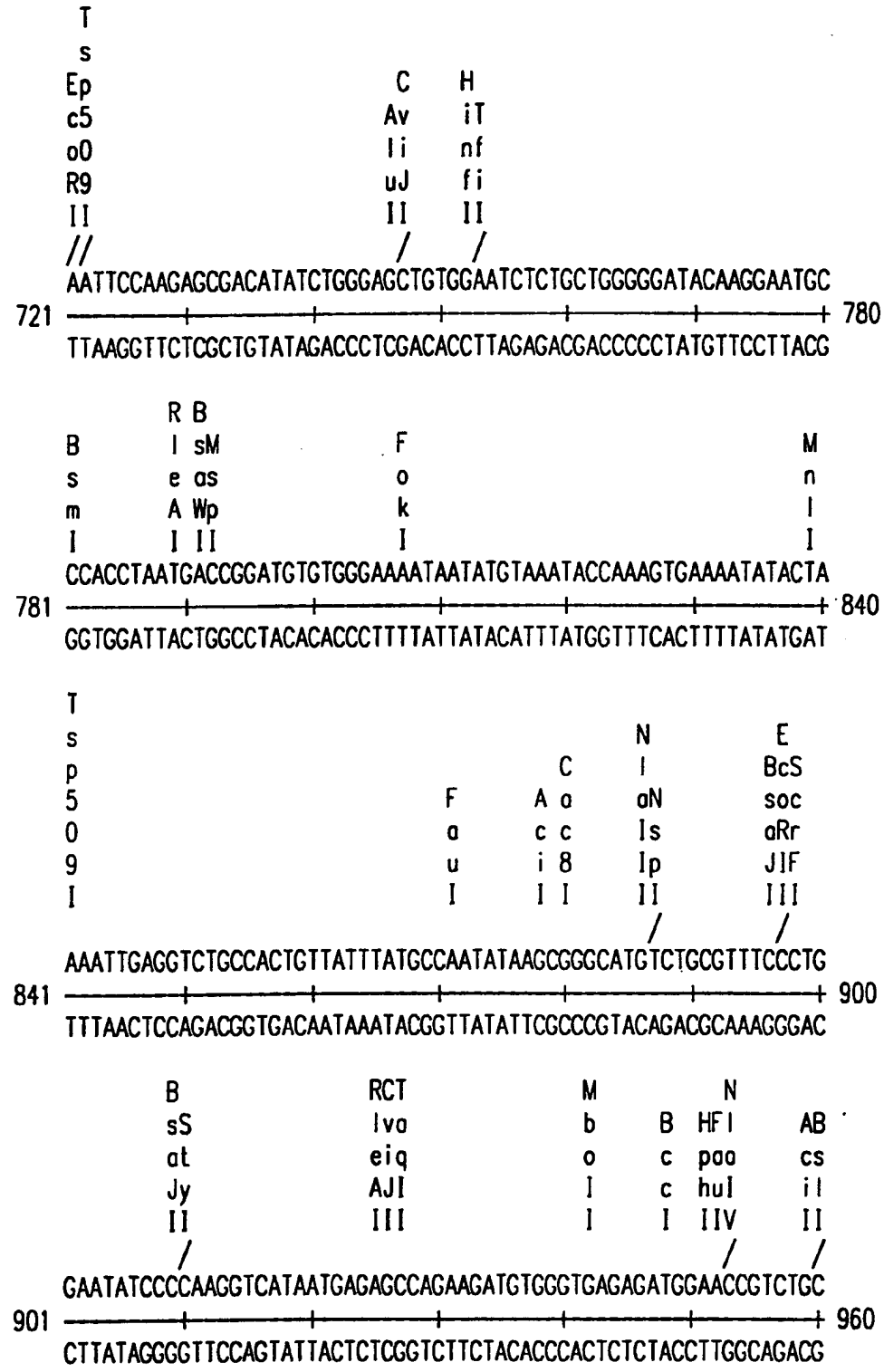
ACAGAGTACTGCAACGGCACATCCGAAGTGTGTGAAGACTTCTTTGTTCAAAACGGTCAC
541
600
TGTCTCATGACGTTGCCGTGTAGGCTTCACACACTTCTGAAGAAACAAGTTTTGCCAGTG
CCATGTGACAATCGCAAGTGGATCTGTATTAACGGCACCTGTCAGAGTGGAGAACAGCAG
601
660
GGTACACTGTTAGCGTTCACCTAGACATAATTGCCGTGGACAGTCTCACCTCTTGTCGTC
TGCCAGGATCTATTTGGCATCGATGCAGGCTTTGGTTCAAGTGAATGTTTCTGGGAGCTG
661
720
ACGGTCCTAGATAAACCGTAGCTACGTCCGAAACCAAGTTCACTTACAAAGACCCTCGAC

FIG.4C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721
780
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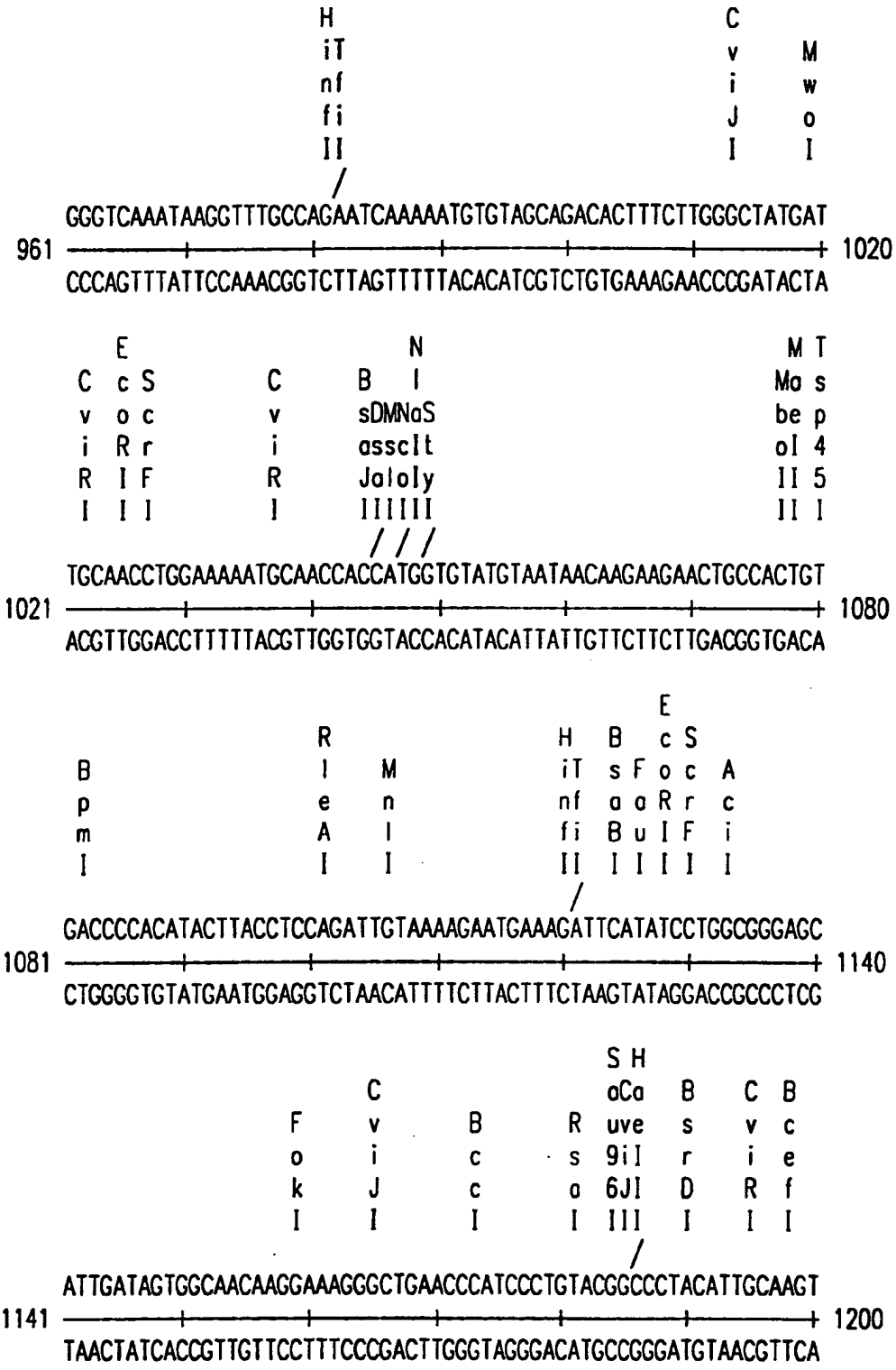
CCACCTAATGACCGGATGTGTGGGAAAATAATATGTAAATACCAAAGTGAAAATATACTA
781
840
GGTGGATTACTGGCCTACACACCCTTTTATTATACATTTATGGTTTCACTTTTATATGAT
AAATTGAGGTCTGCCACTGTTATTTATGCCAATATAAGCGGGCATGTCTGCGTTTCCCTG
841
900
TTTAACTCCAGACGGTGACAATAAATACGGTTATATTCGCCCGTACAGACGCAAAGGGAC
GAATATCCCCAAGGTCATAATGAGAGCCAGAAGATGTGGGTGAGAGATGGAACCGTCTGC
901
960
CTTATAGGGGTTCCAGTATTACTCTCGGTCTTCTACACCCACTCTCTACCTTGGCAGACG

FIG.4D


H
iT
nf
fi
II
C
v
i
J
I
M
w
o
I
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961
1020
CCCAGTTTATTCCAAACGGTCTTAGTTTTTACACATCGTCTGTGAAAGAACCCGATACTA
E
C c S
v o c
i R r
R I F
I I I
C
v
i
R
I
N
B I
sDMNaS
asscIt
JaIoIy
IIIIII
M T
Ma s
be p
oI 4
II 5
II I
TGCAACCTGGAAAAATGCAACCACCATGGTGTATGTAATAACAAGAAGAACTGCCACTGT
1021
1080
ACGTTGGACCTTTTTACGTTGGTGGTACCACATACATTATTGTTCTTCTTGACGGTGACA
B
p
m
I
R
I M
e n
A I
I I
H B E
iT s F c S
nf a a R c A
fi B u I F i
II I I I I I
GACCCCACATACTTACCTCCAGATTGTAAAAGAATGAAAGATTCATATCCTGGCGGGAGC
1081
1140
CTGGGGTGTATGAATGGAGGTCTAACATTTTCTTACTTTCTAAGTATAGGACCGCCCTCG
F
o
k
I
C
v
i
J
I
B
c
c
I
R
s
a
I
S H
aCa B C B
uve s v c
9iI r i e
6JI D R f
III I I I
ATTGATAGTGGCAACAAGGAAAGGGCTGAACCCATCCCTGTACGGCCCTACATTGCAAGT
1141
1200
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FIG.4E

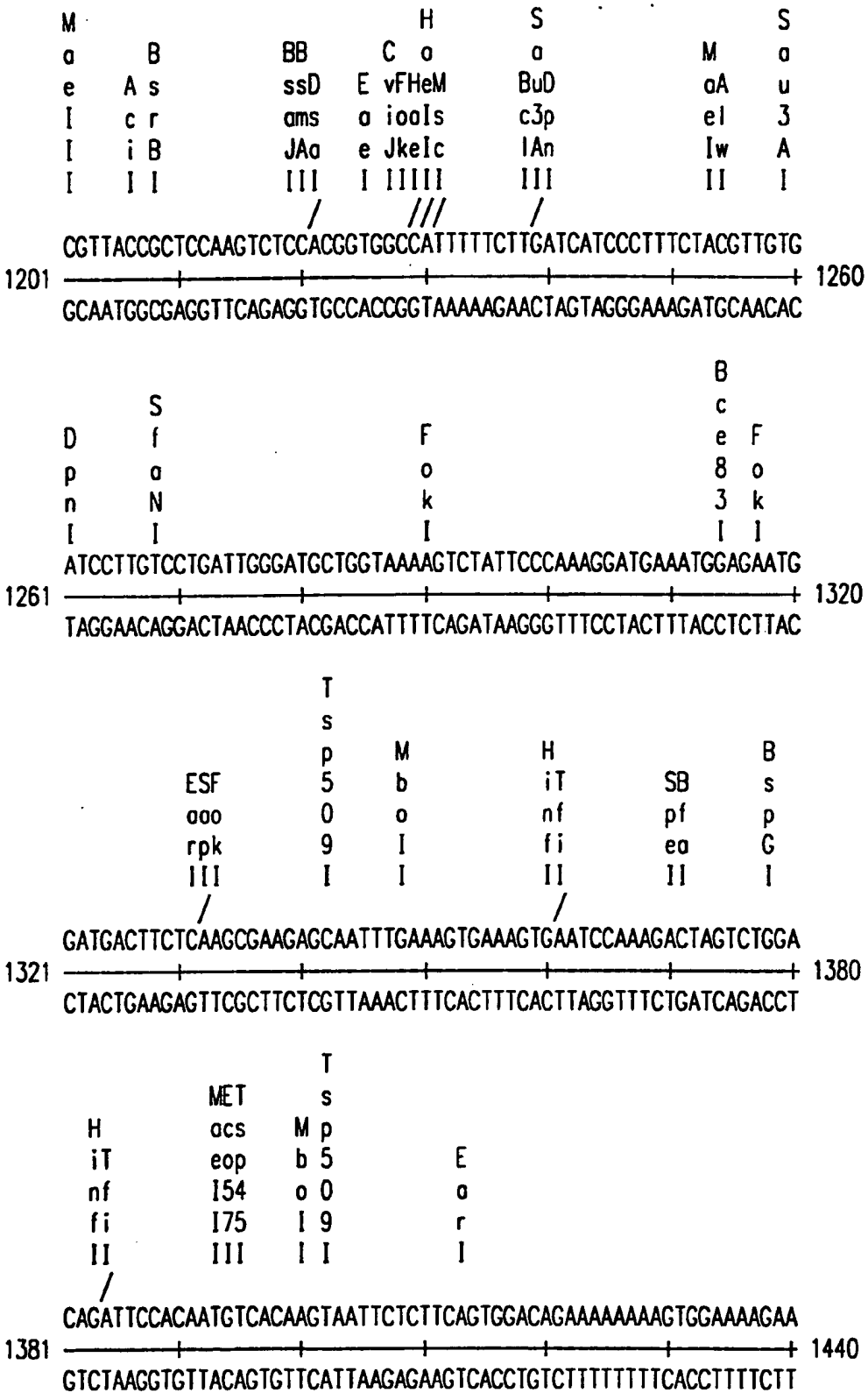

M a e I I I
B A s c r i B I I
BB ssD ams JAa III
C E a e I
H o vFHeM ioolc Jkelc IIIII
S a BuD c3p lAn III
M oA el Iw II
S a u 3 A I
CGTTACCGCTCCAAGTCTCCACGGTGGCCATTTTTCTTGATCATCCCTTTCTACGTTGTG
1201
1260
GCAATGGCGAGGTTCAGAGGTGCCACCGGTAAAAAGAACTAGTAGGGAAAGATGCAACAC
D p n I
S f a N I
F o k I
B c e 8 3 I
F o k I
ATCCTTGTCCTGATTGGGATGCTGGTAAAAGTCTATTCCCAAAGGATGAAATGGAGAATG
1261
1320
TAGGAACAGGACTAACCCTACGACCATTTTCAGATAAGGGTTTCCTACTTTACCTCTTAC
ESF ooo rpk III
T s p 5 0 9 I
M b o I
H iT nf fi II
SB pf ea II
B s p G I
GATGACTTCTCAAGCGAAGAGCAATTTGAAAGTGAAAGTGAATCCAAAGACTAGTCTGGA
1321
1380
CTACTGAAGAGTTCGCTTCTCGTTAAACTTTCACTTTCACTTAGGTTTCTGATCAGACCT
H iT nf fi II
MET acs eop 154 175 III
T s M p b 5 o 0 I 9 I I
E a r I
CAGATTCCACAATGTCACAAGTAATTCTCTTCAGTGGACAGAAAAAAAAGTGGAAAAGAA
1381
1440
GTCTAAGGTGTTACAGTGTTCATTAAGAGAAGTCACCTGTCTTTTTTTTCACCTTTTCTT

FIG.4F

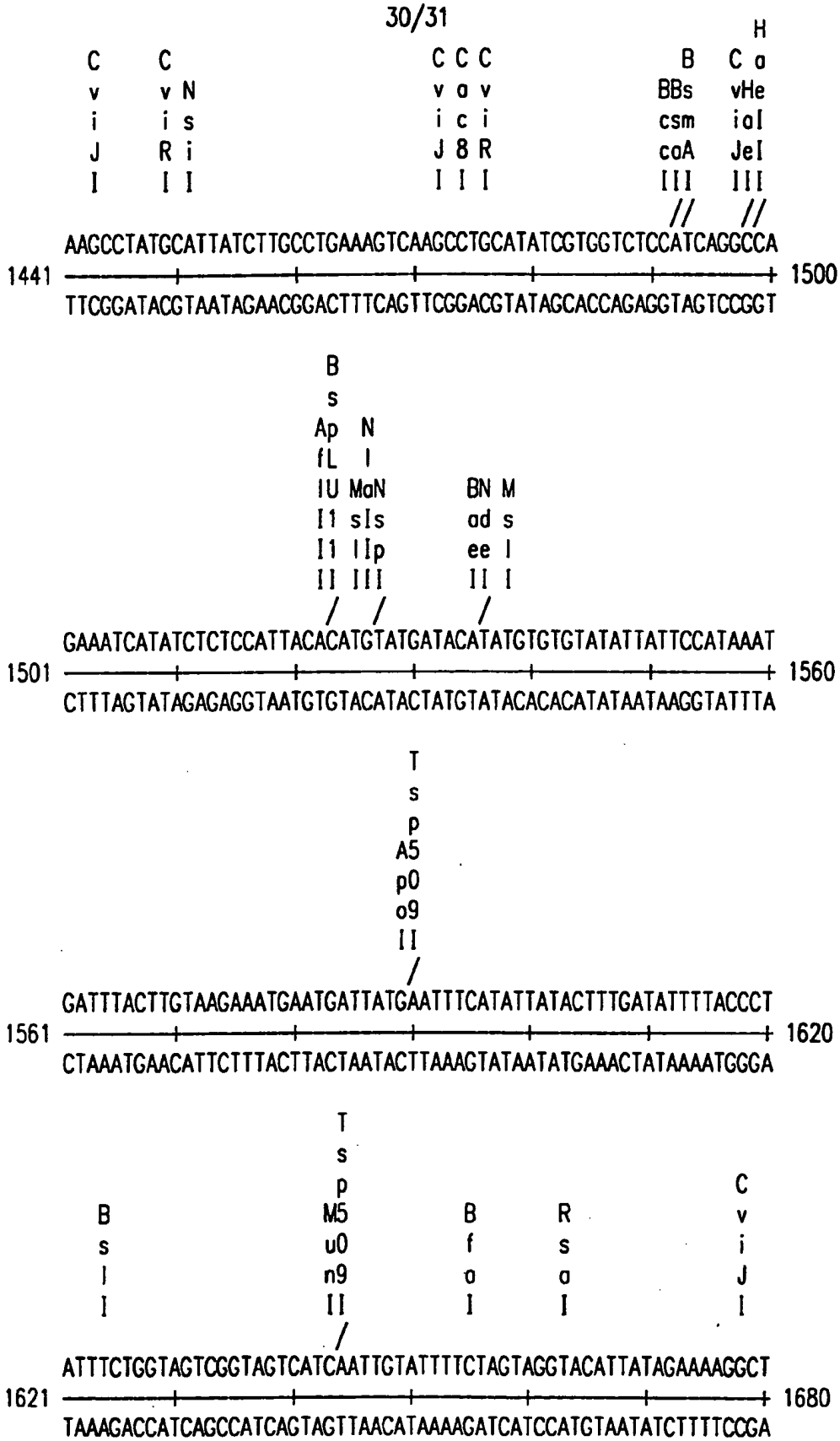
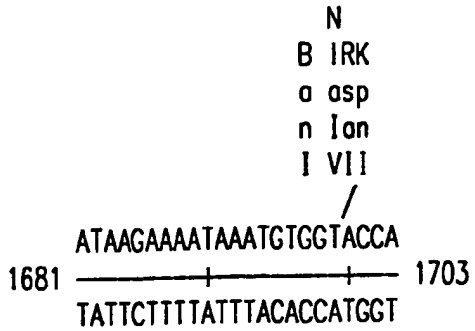

C v i J I
C v i R I N s i I
C C C v v a v i c i J 8 R I I I
B BBs csm coA III
H C a vHe iaI JeI III
// //
AAGCCTATGCATTATCTTGCCTGAAAGTCAAGCCTGCATATCGTGGTCTCCATCAGGCCA
1441
1500
TTCGGATACGTAATAGAACGGACTTTCAGTTCGGACGTATAGCACCAGAGGTAGTCCGGT
B s Ap fL IU I1 I1 II N I MaN sIs IIp III
BN M ad s ee I II I
/ / /
GAAATCATATCTCTCCATTACACATGTATGATACATATGTGTGTATATTATTCCATAAAT
1501
1560
CTTTAGTATAGAGAGGTAATGTGTACATACTATGTATACACACATATAATAAGGTATTTA
T s p A5 p0 o9 II
/
GATTTACTTGTAAGAAATGAATGATTATGAATTTCATATTATACTTTGATATTTTACCCT
1561
1620
CTAAATGAACATTCTTTACTTACTAATACTTAAAGTATAATATGAAACTATAAAATGGGA
T s p M5 u0 n9 II
/
B s I I
B f o I
R s a I
C v i J I
ATTTCTGGTAGTCGGTAGTCATCAATTGTATTTTCTAGTAGGTACATTATAGAAAAGGCT
1621
1680
TAAAGACCATCAGCCATCAGTAGTTAACATAAAAGATCATCCATGTAATATCTTTTCCGA

FIG.4G



Enzymes that do cut:

AciI	AflIII	AluI	AlwI	Alw21I	AlwNI	ApaBI	ApoI
BaeI	BanI	BbsI	BbvI	BccI	Bce83I	BcefI	BclI
BfaI	BglI	BpmI	Bpu10I	Bpu1102I	BsaI	BsaBI	BsaJI
BsaWI	BseRI	BsgI	BsiI	BslI	BsmI	BsmAI	Bsp1286I
BspGI	BspLU11I	BsrI	BsrBI	BsrDI	BstEII	BstYI	Cac8I
ClaI	CviJI	CviRI	DdeI	DpnI	DrdII	DsaI	EaeI
EarI	Eco57I	EcoRI	EcoRII	FauI	Fnu4HI	FokI	HaeI
HaeII	HaeIII	HhaI	HinfI	HphI	KpnI	MaeII	MaeIII
MboII	MnlI	MscI	MseI	MslI	MspI	MunI	MwoI
NciI	NcoI	NdeI	NheI	NlaIII	NlaIV	NsiI	NspI
NspBII	PstI	PvuII	RleAI	RsaI	SapI	Sau96I	Sau3AI
ScaI	ScrFI	SfaNI	SfcI	SpeI	StyI	TaqI	TaqII
TfiI	Tsp45I	Tsp509I	XcmI				

Enzymes that do not cut:

AatII	AccI	AflII	AgeI	Alw44I	ApaI	AscI	AvaI
AvaII	AvrII	BamHI	BanII	BcgI	BcgI	BglII	BsaAI
BsaHI	BscGI	BsiEI	BsiWI	BsmFI	Bsp1407I	BspEI	BspMI
BsrFI	BssHII	Bst1107I	BstXI	Bsu36I	DraI	DraIII	DrdI
EagI	Eam1105I	EciI	Eco47III	Eco105I	EcoNI	Eco0109I	EcoRV
Esp3I	FseI	FspI	GdiII	HgaI	HgiEII	HincII	HindIII
HpaI	MluI	MlyI	MmeI	NaeI	NarI	NotI	NruI
NspV	PacI	Pfl1108I	PflMI	PleI	PmeI	PmlI	PshAI
Psp5II	Psp1406I	PvuI	RcaI	RsrII	SacI	SacII	SalI
SexAI	SfiI	SgrAI	SmaI	SphI	SrfI	Sse8387I	SspI
StuI	SwaI	ThaI	Tth111I	Tth111II	VspI	XbaI	XhoI
XmnI							

FIG.4H

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07295

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/00; C12N 15/12; C12P 21/02
US CL :424/185.1; 435/69.3, 320.1; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1; 435/69.3, 320.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, Derwent, APS, GeneSeq, Swiss Prot, Pir 44, Embl-new6

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 93/25233 (UNIVERSITY OF CONNECTICUT) 23 December 1993, see entire document.	1, 4, 6-19, 21 and 23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 SEPTEMBER 1995	11 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JULIE KRSEK-STAPLES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*